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STUDIES IN THE ANAEMIAS OF INFANCY AND EARLY CHILDHOOD *

PART XII.—THE REGENERATION RATE OF HAEMOGLOBIN AND THE LIFE SPAN OF ERYTHROCYTES IN NORMAL AND PATHOLOGICAL CONDITIONS

BY

H. S. BAAR and T. W. LLOYD

(From the Children's Hospital and the Department of Diseases of Children of the University, Birmingham)

'Haematology will not be an exact science until we can measure the number of red cells, which enter and leave the circulation in the twenty-four hours, with the same accuracy as we can measure the protein metabolism or the work of the heart' (Witts, 1932).

Haematology has for a long time been a purely morphological science. Ehrlich's discovery of specific stains and the perfection of nuclear stains culminating in Pappenheim's panoptic stain opened a vast field for cytological investigation and a generation fascinated by Virchow's cytopathological teaching was confident that the mysteries of the disorders of blood and blood-forming organs are susceptible of solution by a meticulous penetration into the details of the structure of blood cells and their precursors. Not only have such diseases as leukaemia been investigated in this way but affections of the erythron have also been classified from a morphological point of view and hypotheses of their cause and pathogenesis have been based on morphological findings. Much progress has been achieved in this way and many valuable facts established, most of them indispensable for a dynamic consideration of blood diseases.

It has been realized already in this era that anaemia may be due to three main causes: (1) increased destruction, (2) decreased formation of red blood corpuscles (R.B.C.) or their colouring material, (3) a combination of both factors. The possibility of making a quantitative determination of haemoglobin-formation has only been realized in recent years, and the first attempts to solve the problems of anaemia by a metabolic study were made at the other end of the metabolic process, the elimination of iron and the pigmented derivatives of haemoglobin. Since the first quantitative estimations of stercobilinogen by Charnass (1909), Brugsch et. al. (1910–12) and Eppinger and Charnass (1913) a number of investigations on this subject have been published by Lichtenstein and Terwen (1925), Belongowa (1928), Singer (1930), Paschkis (1930, 1933), Josephs (1932, 1934) and especially by Heilmeyer (1931, 1932) and by Watson (1931, 1938). The problem of the rate of haemoglobin-formation, however, has been much less closely studied.

1. The properties of immature erythrocytes in relation to regeneration

The fact that haemoglobin is not introduced into the body as such but is formed within the body from simple materials in the food is the chief characteristic of pigment metabolism. The 'daily haemoglobin intake' must therefore be defined as the amount of haemoglobin delivered in twenty-four hours from the blood-forming organs into the circulation. The possibility of making a quantitative estimation of this amount is raised by the exclusive localization of haemoglobin in the erythrocytes and depends on whether it is possible to distinguish between the young R.B.C. freshly delivered from the haematopoietic organs and mature forms, and also to determine the time taken for these young forms to be transformed into mature erythrocytes. The following forms are generally recognized as immature red cells :

(A) NUCLEATED R.B.C. As there can be no doubt that nucleated R.B.C. are immature forms, the appearance of such cells in the peripheral circulation was for a long time considered as the only reliable sign of increased activity of the erythropoietic tissue. The various forms of erythroblasts, however, appear in the circulation with the exception of the first days of life only in pathological conditions. They are numerous in certain forms of anaemia such as hydrops foetalis, icterus gravis neonatorum, Cooley's anaemia and Jaksch-Hayem's type of erythronoclastic anaemia, whilst in other forms such as acholuric family jaundice, in which an enormous increase in the formation of R.B.C. is proved by several other findings, few if any nucleated R.B.C. may be present. Muir and McNee (1911–12) in a thorough study of experimental anaemia produced by haemolytic serum found that the number of erythroblasts present in the blood 'cannot be said to stand in any definite relationship to the rapidity of blood regeneration,' and one of us (Baar, 1924) has described cases of acute aleukaemic myelosis in which in spite of severe anaemia nu-

* Parts I–XI of these studies appeared in the *Archives of Disease in Childhood*, 1933, **8** ; 1935, **10** and 1937, **12**. Parts XIII and XIV will appear in subsequent issues. References will be printed at the end of part XIV.

cleated R.B.C. were absent from the circulation over the whole period of observation although the bone marrow showed signs of intense erythropoietic hyperactivity. On the other hand, the appearance of numerous nucleated R.B.C. in the peripheral circulation cannot always be looked upon as a sign of intense regenerative activity of the bone marrow but may indicate a simultaneous injury to the precursors of the erythrocyte (erythronoclastic anaemia, Parsons). Bunting (1906) has shown that sudden nucleated red cell crises following the intravenous injection of ricin or saponin in rabbits were accompanied by signs of bone marrow injury, as shown by pyknosis and fragmentation of the nuclei, which in the case of saponin may be followed by a sclerosis of bone marrow tissue. It would, however, be erroneous to exclude increased formation of R.B.C. when numerous nucleated red cells are present in the circulation. Parsons (1938) has brought forward a series of arguments proving the regenerative nature of erythroblastaemia in Cooley's anaemia and erythroblastosis foetalis. The coexistence of nucleated red cells and reticulocytosis is rightly called by Witts (1932) an ' unimpeachable witness of an accelerated delivery of red cells into the circulation.' It is doubtful, however, if a transformation of nucleated R.B.C. into mature erythrocytes takes place in the peripheral circulation. In any case physiological regeneration of R.B.C. takes place without the delivery of nucleated R.B.C. The number of erythroblasts therefore cannot either in normal (newborn) or in any pathological condition form a basis for the quantitative estimation of the regeneration rate.

(B) HOWELL-JOLLY BODIES, CABOT'S RINGS and SCHLEIP'S LOOPS are certainly, azurophilic stippling and Schilling's ' erythroconten ' probably, fragments of the nuclear substance. Not one of these forms is a necessary stage in the maturation of erythroblasts. Furthermore by special stains (methylene-blue-picric acid) nuclear fragments have been shown in every red cell (Kronberger, 1912; Gruner, 1913) and must therefore be disregarded in a quantitative consideration of blood formation.

(C) BASOPHILIC STIPPLING. Originally described as a degenerative change of erythrocytes due to intoxications, particularly lead poisoning (Grawitz, 1899, 1900), it is to-day generally recognized as a sign of immaturity (Sabrazès, 1910; Lutoslawski, 1904 ; Naegeli, 1931 ; Walterhöfer, 1913; Hertz, 1910; Schilling, 1911, 1913). Though some authors considered it as a product of nuclear disintegration (Schmidt, 1909 ; Kreibich, 1921; Koch, 1924) it has been proved by Hawes (1909), Key (1921), Whitby and Britton (1933) that basophilic stippling is a particular manifestation of the same basophilic material as the polychromatophilic and reticular substance. After incubating blood containing many reticulocytes for some days we have repeatedly observed basophilic stippling in blood from which it was absent before; in this case it was obviously due to degeneration of the reticular substance. This in connexion with the experiments of Key (1921) and with the fact that erythrocytes with basophilic stippling occur only in some forms of anaemia leads to the conclusion that basophilic stippling is a sign of pathological regeneration. According to Whitby and Britton (1933) stippling is a minor change of the polychromatophilic material of young red cells, the change taking place in the bone marrow. In estimating blood regeneration only those findings can be taken into account which are constant in normal and pathological young red cells; hence basophilic stippling must be disregarded.

(D) REFRACTILE GRANULES. Two types of refractile granules have been described by Isaacs (1924, 1938) as well as two types of non-refractile granules, and are regarded by him as a sign of immaturity in erythrocytes. This author has observed such granules in large numbers after blood transfusion and considers them as forming an intermediate stage between reticulocytes and mature R.B.C. As Isaacs claims that erythrocytes can be delivered from the bone marrow in this stage their enumeration would appear to be necessary for the determination of the regeneration rate. In our experiments on the maturation of reticulocytes in vitro, which are described below and in which a vital transformation of reticulocytes into mature erythrocytes has been shown beyond question, no such intermediate stage with refractile granules has been observed; but if blood is incubated for more than twenty-four hours many refractile bodies may be seen, not only in erythrocytes containing haemoglobin, but also in ghosts and at the same time basophilic stippling and basophilic spherical bodies, corresponding to Heinz-bodies, the ' blue granules ' in toxic anaemia, become evident in stained films. These refractile bodies may, in spite of some differences in the description, correspond to those found by Pepper (1922) in incubated blood and which he regarded as identical with the substantia metachromatica of Demel. Their appearance under the conditions mentioned above strongly supports the opinion that they are degenerative products in young erythrocytes. Heath and Daland (1930) also observed refractile bodies in incubated blood which, in stained films, appeared as basophilic spots or rods; they considered these bodies to be identical with basophilic stippling and Schilling's ' erythroconten.' Here apparently different things are confused, for ' erythroconten ' do not appear as basophilic (orthochromatic) but as azurophilic (basophilic metachromatic) rods. Isaacs believes that the refractive bodies of Heath and Daland are different from those described by himself. Refractile bodies corresponding to Isaacs' description of type 1 granules have been observed by us in the fresh blood in icterus gravis neonatorum. They were numerous and distinct in ghosts when the cells were haemolysed in vitro by a solution containing saponin, formalin and brilliant cresyl blue (Baar's platelet counting fluid). Often two granules were present, one larger than the other, and the granules often showed a ' dancing ' movement in the erythrocyte stroma. It cannot be said, however, that this finding is constant in cases with high reticulocytosis.

Isaacs' opinion that numerous immature R.B.C. appear in the circulation after blood transfusions has recently been criticized by Maizels and Paterson (1940), whose view agrees with our experience of a series of clinical observations and with the results of animal experiments. Robertson (1917) after repeated small blood transfusions in rabbits observed a marked decrease or even complete disappearance of reticulocytes in thirteen out of sixteen experiments and only found an increase in one. Hess (1909) and Itami (1908) report depression of the bone marrow activity in experimental plethora. Vogel and McCurdy (1913), Lee, Minot and Vincent (1916) observed a considerable drop in the number of reticulocytes besides other signs of depressed bone marrow activity following transfusions in pernicious anaemia. Moldawsky (1928) after transfusions in various anaemias of childhood observed a considerable decrease in the number of reticulocytes. An exceptional case when, following transfusions in a haemolytic anaemia of the newborn, the reticulocytes disappeared practically completely from the circulation and the baby's macrocytic red cell population was completely replaced by the normocytic cells of the donor and a case of acholuric family jaundice in which after two blood transfusions the reticulocytes were absent from the peripheral blood for a period of six days will be reported in part XIV. The opposite reaction, as described by Friedlander and Wiedemer (1929) in two cases of duodenal ulcer and in a few cases of pernicious anaemia by Belongowa (1928) has been also met by the writers but is a rare event. Considerable increases in the numbers of reticulocytes after blood transfusions observed by Denecke (1923) were due to incompatible blood.

From the foregoing discussion it can be concluded that though refractile granules can be found in young erythrocytes the regenerative nature of such granules is not proven, that similar granules may indicate degeneration and that a stage with refractile granules is not obligatory in the transition between reticulocytes and mature erythrocytes. It must be further taken into account that Schilling (1911, 1924) found in R.B.C. of malarial blood two minute highly refractile granules which he was later able to detect in normal erythrocytes and which he considers to be centrioles. For type 1 of his granules Isaacs mentions that ' in the normoblast they have a position by the nucleus suggesting a centrosome-like body or more nearly the centrosphere.' For all these reasons the enumeration of R.B.C. with refractile granules cannot be considered as a feasible way of estimating blood regeneration.

(E) POLYCHROMASIA. The abundance of polychromatophilic erythrocytes after haemorrhage and their occurrence in the bone marrow and foetal liver are sufficient proof that polychromasia is a sign of immaturity of erythrocytes. From the investigations of Biondi (1908), Schilling (1911b, 1913), Hawes (1909), Hertz (1910), Key (1921), Arrak (1927), Simmel (1925), Seyfarth (1927), Heath and Daland (1931) and Whitby and Britton (1933) it may be concluded that polychromasia in stained films is a manifestation of that basophilic substance which in supravital staining appears as a reticulo-granular structure. The conception of Key (1921), however, that this basophilic substance is diffuse in the plasma of R.B.C. and that the reticular structure is a result of precipitation by the stain, and the similar idea of Watson and Clarke (1937) who suggested that the reticulum is due to precipitation of the erythrocyte's porphyrin by brilliant cresyl blue, must be abandoned since Simmel (1925) and Seyfarth and Jürgens (1927) have seen a reticulum in unstained films examined in the dark field and Simmel has been able to remove the reticulum from fresh red cells by means of a micromanipulator. It must therefore be concluded that the reticular substance is genuine and polychromasia is the result of dissolution of this substance in alcohol with subsequent diffuse imbibition of the cytoplasm. Ferrata and Boselli's claim (1910) that reticulocytes and polychromatophilic erythrocytes can be demonstrated in the same film, if the supravital staining is followed by fixation and counterstaining with Pappenheim's method, is certainly erroneous. If perfectly reliable methods are used for supravital staining polychromasia is absent in counterstained films. If, however, a layer of brilliant cresyl blue on a slide is used for supravital staining the simultaneous presence of reticulocytes and polychromatic erythrocytes is occasionally observed, but in such films the indistinctness and haziness and the pale blue colour of the reticulum are always signs of an imperfect technique.

As a rule polychromasia may be considered as a sign of erythropoietic activity and this phenomenon was the first morphological property of R.B.C. used for a rough estimation of the regeneration rate. Schilling (1924, 1926) made use of his ' thick drop ' method and estimated the erythropoietic activity from the average number of polychromatophilic erythrocytes in one microscopic field. If, however, the regeneration rate is to be determined for metabolic studies a method based on the enumeration of polychromatophilic erythrocytes must be rejected for the following reasons:

(a) If reticulocytes are counted after supravital staining and polychromatophilic R.B.C. in a film stained with Leishman's stain or with Jenner-Giemsa in the same blood sample the number of polychromatic R.B.C. is always lower than the number of reticulocytes. The few observations with higher figures for polychromatic R.B.C. than for reticulocytes were found in a paper by Denecke (1923), whose figures for reticulocytes, however, are often exceptionally low.

There is no fixed relation between the number of polychromatic erythrocytes and reticulocytes. This is evident from table 1.

The relation between reticulocytes and polychromatic erythrocytes (R/P) varies here between 1·53 and 10·5. The figures are particularly high in cases with low reticulocyte counts. On the other hand, in the case of high reticulocyte counts as in experimental lead poisoning and phenylhydrazin anaemia Whitby and Britton (1933) have shown that

the sum of polychromatic and stippled cells is only little below the number of reticulated cells and that the two figures run fairly parallel. The relation between the number of reticulocytes and the sum of polychromatic and stippled cells in these experiments was about 1·2–1·5. It can be concluded that whilst even minute amounts of basophilic substance can be demonstrated by supravital staining a considerably higher amount is necessary to cause polychromasia.

TABLE 1

RELATION OF RETICULOCYTES TO POLYCHROMATOPHILIC ERYTHROCYTES IN TWENTY CASES OF HAEMOLYTIC ANAEMIA

No.	RETICULOCYTES PER CENT.	POLYCHROMATIC R.B.C. PER CENT.	R/P
1	27·9	16·2	1·72
2	19·6	7·1	2·76
3	18·4	4·1	4·50
4	18·0	11·8	1·53
5	13·2	6·4	2·06
6	11·4	5·6	2·04
7	10·8	2·7	4·00
8	9·0	2·3	3·92
9	8·4	2·1	4·00
10	8·2	1·8	4·57
11	6·6	4·2	1·57
12	5·4	2·8	1·93
13	5·1	2·0	2·55
14	5·1	2·4	2·12
15	4·8	2·4	2·00
16	2·3	0·8	2·88
17	2·1	0·2	10·50
18	1·8	0·2	9·05
19	0·7	0·1	7·00
20	0·5	0·1	5·00

(b) The close relation between regeneration and polychromasia does not exclude the possibility that a similar change in the staining property of erythrocytes can be caused by degeneration. This has been claimed by Bodon (1903), Hirschfeld (1909) and Naegeli (1931) but denied by Heinz (1908) and Arrak (1927). Biondi (1908), who was the first to give proofs for the identity of the basophilic and reticular substance, admits the possibility of degenerative polychromasia. We have repeated Bodon's experiments. Normal human blood was incubated in sealed capillary tubes for several days. At intervals of twenty-four hours films were made and stained with Leishman's stain, Jenner-Giemsa and Manson-Koch's borax-methylene blue. After seventy-two to ninety-six hours most of the red cells showed a vacuolar degeneration, a greyish staining with Leishman and Jenner-Giemsa and a deep blue colour with Manson-Koch's stain. These are, of course, extremely unphysiological conditions, but the results show that the possibility of a degenerative polychromasia cannot be ignored.

(F) RETICULOCYTES. Since Ehrlich and Howell described basophilic nets in unfixed R.B.C. stained with basic stains many investigations have been published on this subject. The old conception of Chauffard and Fiessinger (1907) that the reticular substance is a degenerative phenomenon and that increased fragility is the result ' d'une lésion granuleuse special des hématies ' is to-day definitely dismissed. As a result of investigations of Hawes (1909), Schilling (1911, 1913), Hertz (1910), Key (1921), Seyfarth (1927), Moldawsky (1928) and Naegeli (1931) the regenerative nature of reticulocytosis, first suggested by Smith, was generally recognized. The finding of Seyfarth (1927) that in early foetal life, when megaloblasts are the only form of R.B.C., all these cells contain a vitally staining reticular substance is especially convincing. The extensive investigations of this author prove beyond question that the reticulocyte is an inevitable transitional stage in the normal development of R.B.C., but there is still disagreement as to whether all R.B.C. delivered from the bone marrow contain reticular substance or not. This question is a fundamental one, as only if this be so is a calculation of the regeneration rate possible on the basis of reticulocyte counts. Isaacs claims that R.B.C. enter the circulation not only in the state of reticulocytes but also as R.B.C. with refractile granules. His proofs, as has been pointed out above, are not convincing. Minot and collaborators (1928) conclude from their investigations on blood regeneration in pernicious anaemia treated with liver extract that ' the erythrocyte increase at the time of the peak of the reticulocyte rise is almost entirely to be accounted for in the cases with less than 2·5 millions R.B.C. by reticulocytes ' but ' when the R.B.C. count is relatively high the entire increase in erythrocytes at the time of the peak of the reticulocyte rise, apparently, is not due to these young forms.' This conclusion is based on the following observation: In a series of eighty-nine cases in which the observed concentration of reticulocytes at the peak of reticulocyte response, Epr (where Ep is the number of erythrocytes at the peak and r the percentage of reticulocytes at the same time) was compared with the concentration of reticulocytes at the peak of the rise as calculated from the formula $\frac{Eor}{1-r}$ (where Eo is the number of R.B.C. before liver treatment). A good agreement was found only in cases with low initial erythrocyte counts. It can easily be seen that $Epr = \frac{Eor}{1-r}$ when $Ep - Eo = Epr$. This means that all reticulocytes delivered from the bone marrow from the start of liver treatment are present at the peak of reticulocyte response still in the reticulated stage and that no destruction of R.B.C. had taken place in this period. Actually there is an intense destruction of erythrocytes at the peak of reticulocyte rise and before, as will be shown later [cf. Heilmeyer (1932), Watson (1931, 1938), Belongowa (1928), Dobriner and Rhoads (1938)] and the life span of the erythrocyte at this time is very short. The close agreement between the observed and calculated figures in cases with low initial erythrocyte counts shows that in these cases the two factors, maturation of reticulocytes into non-reticulated R.B.C. and the destruc-

tion of erythrocytes, are well balanced. It can be easily conceived that the increase in the number of R.B.C. at the peak of the reticulocyte response will the more closely correspond to the number of cells just delivered from the bone marrow the shorter the duration of life of the erythrocyte or the severer the disease. With the figure Eo approaching O, Ep will approach Epr and with the shortening of the erythrocyte's life span the latter will approach the maturation time of reticulocytes. The argument of Minot and his collaborators cannot be accepted therefore and their important observations may be given a different explanation.

From investigation on blood regeneration in pernicious anaemia came the first proof that there is a fixed mathematical relation between the number of reticulocytes and the delivery of R.B.C. from the blood-forming organs. Riddle (1930) was the first to apply Robertson's (1923) formula for the kinetics of growth to blood regeneration in pernicious anaemia. In Robertson's fascinating book innumerable proofs are given that in the whole of living nature growth follows the rules of the autocatalyzed reversible reaction. Such a reaction is determined by the formula $\log \frac{x}{a-x} = ka(t-t)$. 'As applied to the increase in the concentration of erythrocytes during the reticulocyte response, the value (x) in this equation represents the increase in the concentration of erythrocytes after any number of days of treatment (t). The value (a) is a constant representing the total increase in the erythrocyte concentration during the reticulocyte response, and as blood regeneration takes place during the reticulocyte response (x) approaches (a) as a limit. The value (t) represents the number of days of treatment when $x = \frac{a}{2}$, which usually is near the peak of the reticulocyte response. The value (k) is a constant which is determined for each different application of the formula.' It has been shown that for each case the same formula can be applied for the total increase of R.B.C. and for the increase of non-reticulated cells as well, the only difference being in the value (t). Furthermore if from a formula differing only in the figure (a) the numbers of reticulocytes per c.mm. liberated from the bone marrow are calculated and a corresponding 'differential' curve constructed the latter shows a satisfactory agreement with the observed numbers of reticulocytes. These investigations afford evidence that if erythrocytes are not all liberated from the bone marrow as reticulocytes there is at least a fixed mathematical relation between the daily intake of R.B.C. and the number of reticulocytes. Complete evidence that all erythrocytes delivered from the bone marrow are reticulocytes would be supplied if it were possible to show that the bone marrow, or more exactly the haematogenic intersinusoid capillaries, contain only reticulocytes. In man such investigations meet with insurmountable difficulties. Post-mortem examinations are useless; sternal puncture always yields a mixture of bone marrow and circulating blood and besides this there are difficulties in the supravital staining of bone marrow. Only staining in a solution of brilliant cresyl blue in normal saline with the addition of sodium citrate gave in our experience satisfactory results. It can be shown in that way that rats and guinea pigs with 1 to 2 per cent. reticulocytes in the circulating blood have about 50 per cent. reticulocytes in the bone marrow. The non-reticulated red cells may be due to an admixture of circulating blood. We have tried to wash out the circulating blood by opening the right auricle and injecting normal saline into the aorta from the left ventricle in guinea pigs. For reasons unknown the subsequent supravital staining of the bone marrow was not always successful and only once was a film obtained in which all R.B.C. contained reticulum. For rabbits Istomanowa (1926) succeeded in showing that all the red cells of the bone marrow are reticulated. In order to elucidate the question whether in human beings all erythrocytes entering the peripheral circulation from the bone marrow contain supravitally staining substance the excretion of bile pigments has been determined in three cases of aleukaemic myelosis without any reticulocytes in the circulating blood and in one case of acholuric jaundice where two blood transfusions were followed by a six days' period of areticulocytosis. The idea which led to this investigation was that if the assumption that the total intake of R.B.C. is due to a liberation of reticulocytes from the bone marrow be correct the absence of such cells from the peripheral circulation must mean that there is no replacement of destroyed erythrocytes at all. As there is no evidence for the storage of bile pigments, and the reutilization of pigments can be excluded in such cases,* the amount of bile pigment excreted must correspond to the fall in the total circulating haemoglobin. The methods applied at the present time for stercobilin or stercobilinogen determinations are useless for this purpose. It is recognized by many authors that only part of the excreted bile pigment is estimable by stercobilinogen determinations. Greppi (1926, 1930) assumes that the real destruction of haemoglobin is twice that calculated from stercobilinogen determinations while according to Eppinger (1920) and Schiødt (1938b) the latter figure is only a fourth of the true one. One-fourth to one-fifth is the figure calculated from the stercobilin excretion in stools and bile pigment excretion in bile fistula dogs if that decrease is taken into account which takes place if the bile is not returned to the intestine. The bile pigments of human stools have been investigated by one of us (H.S.B.) in collaboration with Dr. E. Hickmans. As the result of these investigations a method for estimation of the 'total bile pigments' has been elaborated. These investigations are not completed yet and will be published later (Baar and Hickmans,

* There is much controversy about the reutilization of bile pigments since the classical experiments of Stadelmann (1871). It is denied by Whipple and his collaborators from experiments on dogs. From investigations on pigment-excretion which will be reported later it was concluded that there is no reutilization in normal and in many pathological conditions but that it may occur to a considerable extent in acholuric jaundice and some other haemolytic anaemias.

1941). It is possible here only briefly to outline the method and its experimental foundation.

A study of the absorption curves of about two thousand acid alcoholic stool extracts combined always with spectroscopic and in many instances with fluoroscopic examination gave evidence that besides stercobilin, copromesobiliviolin and copronigrin (Watson, 1932, 1934) there is at least one pigment present with an absorption increasing between 490 and 420 mμ. Depending on the relation between stercobilin and this pigment, not on the amount of copromesobiliviolin, the extracts show with zinc alcohol either a green fluorescence all through or a green fluorescence in the upper layer and a reddish-yellow fluorescence in the lower layer, or even a reddish fluorescence only when examined in the dark box devised by Elman and McMaster (1925). By incubation of stool emulsions a striking change of the absorption curve has often been obtained, indicating a transformation of stercobilin into the above mentioned pigment or pigments. In a few instances the reverse process has been observed. By fractional extraction and chromatographic separation two new pigments have been isolated: a red one crystallizing in needles and a brownish-yellow obtained up to the present moment only in amorphous form. The latter pigment has been reduced by means of sodium amalgam into a leuko-compound giving a weak Ehrlich-reaction. Both pigments show similar absorption curves between 490 and 420 mμ but differ in the region 520 to 490 mμ. ' Stercobilin ' prepared according to Garrod and Hopkins' method (modification of Elman and McMaster) proved to be an impure mixture of stercobilin and these two pigments (stercofulvin and stercorubrin). Analysis of absorption curves of stool extracts indicates that either one of these pigments is prevalent or both run parallel. This justifies a calculation on the basis of two pigments. The validity of the Beer-Lambert law has been ascertained (only within certain limits of concentration) for these pigments and pigment mixtures in the spectral regions in which the determinations are made (490 and 440 mμ). Crystalline stercobilin has been prepared by the methods of Watson (1934) and of Heilmeyer and Krebs (1934), and several crystalline mixtures have been obtained containing stercobilin and stercofulvin (and/or stercorubrin). The absorption relations have been determined for pure crystalline stercobilin and for crystalline mixtures. The quotient E490/E440 has been determined in a solution of stercofulvin free from stercobilin. Thus although neither stercofulvin nor stercorubrin have been isolated in pure form it was possible with the use of the Vierodt formula to calculate the absorption relations of stercofulvin (and/or stercorubrin) for the spectral region of filters 490 and 440 in the Evelyn photoelectric step photometer. With the known A values for stercobilin and stercofulvin (and/or stercorubrin) the amount of both pigments can be calculated from the extinctions at 490 and 440 mμ by means of the Vierodt formula. This gives approximately the amout of ' total bile pigments ' if copromesobiliviolin is absent or present in traces only, which can be ascertained by spectroscopic examination. A chart has been constructed in which the quotient E490/E440 was plotted against the constants for calculation of ' total bile pigments ' (A-values for mixtures of stercobilin plus stercofulvin and/or stercorubrin). Copronigrin, the relationship of which to bilirubinoid pigments is not settled yet and which can be disregarded at least in stools freshly extracted, was eliminated by treatment with zinc acetate.

Case 1. (R. A.) a boy of three years was admitted to the Children's Hospital, Birmingham, under the care of Professor Parsons on February 28, 1940. He had been treated for streptococcal otitis media in September, 1939, when he was anaemic, and had bruises over the iliac crests. The aural discharge had persisted despite treatment. Five days before admission he suddenly became ill and rather blue. On admission there was severe anaemia and enlargement of cervical and axillary glands. His W.B.C. counts were between 2100 and 4000, 80 to 90 per cent. being lymphoid cells. Some of these had the appearance of mature lymphocytes, some of micromyeloblasts. The platelets were always few. Reticulocytes were seen only on one occasion when 0·05 per cent. were counted. In many other examinations no single reticulocyte was found. A sternal puncture yielded a thin fluid containing a few cells of which all white cells were pathological myeloblasts (paramyeloblasts) and not a single nucleated R.B.C. was seen. Stools were collected over a period of six days and estimations of bile pigments were made in triplicate. Only two haemoglobin determinations were made with Evelyn's photoelectric step photometer (standardized by van Slyke's oxygen capacity method) in an interval of three days between two blood transfusions. Blood volume was calculated from the body weight on the basis of Seckel's (1930, 1936) figures. The results of the investigations on this and the two other children are given in table 2. The average daily wastage of haemoglobin was calculated from the daily excretion of bile pigment on the basis that 100 mgm. of pigment correspond to 2·5 g. haemoglobin.* The figures show good agreement between the excretion of pigmented haemoglobin derivatives and the drop in the total circulating haemoglobin. It can be concluded therefore that in this case in which reticulocytes were absent no regeneration of R.B.C. took place.

Case 2. D. W., a girl of three and a half years, was admitted under care of Dr. Braid at the Children's Hospital, Birmingham, on June 11, 1940. She had been fairly well until the beginning of June, 1940, when she began vomiting, developed a cough and became pale. On admission enlargement of liver and spleen and bruises on both legs were found. The haemoglobin was 42 per cent. (Haldane),

* This calculation is used by most authors working on pigment excretion. It is based on the molecular weight of haemin which is 651. The molecular weight of the prosthetic group of haemoglobin (proto-haem) is 616 and that of bilirubin 582. It is therefore correct when Hawkins and Whipple (1938) take 35 mgm. bilirubin as equivalent to 1 gm. haemoglobin. In our method, however, the calculations are based on an absorption relation determined for stercobilin hydrochloride, the molecular weight of which is 630. The calculation 40 mgm. stercobilin = 1 gm. haemoglobin is therefore approximately correct.

R.B.C. 2,470,000 per c.mm., reticulocytes 0·32 per cent., W.B.C. 3100 per c.mm. with 82 per cent. lymphoid cells, at least 23 per cent. of which were myeloblasts. Occasional neutrophilic myelocytes and metamyelocytes were found, and 2 to 6 normoblasts per 100 W.B.C. A sternal puncture showed an increase in the number of myeloblasts (23 per cent.) and a low count of nucleated R.B.C. (6 per cent.). On June 30, 1940, the child was taken home; she was readmitted on September 6, 1940. The findings at this time were similar to those of June except in the complete absence of reticulocytes from the circulating blood. Only on one occasion was a single reticulated R.B.C. seen. The child died on September 14, 1940. Post-mortem examination (H. S. B.) showed a severe anaemia; subpleural and subepicardial haemorrhages, enlargement of lymph nodes, liver and spleen, leukaemic ulcers in the lower ileum and haemosiderosis of the liver. Histological examination revealed a high degree of myeloid metaplasia in liver, spleen, kidneys and lymph nodes. In the liver there was cellular infiltration in the interlobular spaces and clusters of cells in the liver capillaries as well. The bone marrow was cellular, most of the cells being myeloblasts; only occasionally a few neutrophilic and eosinophilic leucocytes were present. Nucleated R.B.C. were encountered only exceptionally in the bone marrow and were altogether absent from the foci of myeloid metaplasia. Determinations of bile pigments in the stools were made over a period of six days of the 'a regenerative' stage, three blood counts were done during this period, and the results are shown in table 2.

Case 3. G. P., ten months old, was from May 25, 1941 to June 11, 1941 an inpatient in the Children's Hospital, Birmingham, under care of Professor Wilkinson, for upper respiratory tract infection, gastro-enteritis and haemolytic anaemia. Neither blood picture nor bone marrow (sternal puncture) were at that time suggestive of leukaemia. On July 17, following first diphtheria inoculation, the child was noticed to have progressive loss of appetite. She had been also noticed to go yellow. On readmission (July 22, 1941) she was pale, purpuric spots were seen all over the body and an ulcer in the mouth. All W.B.C. were lymphoid forms, many of them immature. No nucleated R.B.C. and no reticulocytes were found in the peripheral circulation. Sternal puncture: all nucleated cells were pathological, immature lymphoid cells (? micromyeloblasts); nucleated R.B.C. absent. Stools were collected and bile pigment estimations done over two periods of three and two days respectively. Three blood counts were made between July 24 and August 3, 1941. The results are shown in table 2. The child died on August 3, 1941. The post-mortem examination (H. S. B.) showed ulceration of the left tonsil and of oesophagus, lobular pneumonia, purulent otitis media, general anaemia, enlargement of liver, spleen and kidneys, the latter with diffusely outlined greyish-white areas and numerous haemorrhages.

Case 4. R. T., a boy of seven years, admitted on June 9, 1941, to the Children's Hospital, Birmingham, under the care of Dr. Neale. He was a typical case of acholuric family jaundice; a brother died at the age of seven months from severe anaemia, purulent otitis media and glomerulonephritis, two sisters had their spleens removed. On admission the haemoglobin was 5·5 gm. per cent. and the red cell count 2,030,000 per c.mm. On the next day the haemoglobin was 4·42 gm. per cent. and the red cell count 1,700,000 per c.mm. Following two blood transfusions the reticulocytes disappeared completely from the peripheral circulation and were absent for six days. At the end of this period the haemoglobin was 7·0 gm. per cent. and the red cell count 2,550,000 per c.mm. The calculated blood volume was 2000 c.c. The amount of transfused

TABLE 2

BILE PIGMENT EXCRETION AND FALL IN HAEMOGLOBIN IN THREE CASES OF ALEUKAEMIC MYELOSIS WITH AREGENERATIVE ANAEMIA

Name	Date	Haemoglobin gm. per cent.	Blood volume c.c.	Total circulating haemoglobin (gm.)	Total bile pigment excretion (mgm.)	Average daily wastage of haemoglobin (gm.)	Average daily drop in total circulating haemoglobin (gm.)
R. A.	4.3.40	4·95	1334	66·0			
	6–7.3.40				204		
	7.3.40	3·62		50·0			
	7–11.3.40				828		
	11–12.3.40				214	5·2	5·3
D. W.	7.9.40	3·85	1120	47·3			
	8–11.9.40				370		
	13.9.40	2·34		26·2			
	11–14.9.40				276		
						2·58	2·92
G. P.	24.7.41	7·6	542	41·2			
	24–27.7.41				214		
	30.7.41	4·97		26·9			
	30.7–1.8.41				162		
	3.8.41	3·72		20·2			
						1·88	2·10

haemoglobin was approximately 128 gm. The difference in the amount of circulating haemoglobin between the starting and end-points of the aregenerative phase corresponded to a gain of 51·6 gm. 76·4 gm. haemoglobin had been lost therefore in that period. The amount of excreted bile pigment over the same period was 2872 mgm. corresponding to haemoglobin wastage of 71·7 gm.

In all four cases there is a fairly close agreement between the actual loss of haemoglobin and that calculated from the bile pigment excretion, showing that in the absence of reticulocytes there is no regeneration of destroyed R.B.C.

From the foregoing observations it may be concluded that R.B.C. are liberated from the bone marrow only in the stage of reticulocytes.

With regard to this conclusion a modification of Witts' schematic diagram of the erythron is proposed. In this diagram the independence of the normoblastic generation from the megaloblastic one and the possibility of extravascular formation of proerythroblasts (Naegeli, 1931; Turnbull, 1934; Gilmour, 1941) is taken into account as well as the delivery of red cells from the erythrogenic intersinusoid capillaries into the peripheral circulation exclusively in the reticulated stage (fig. 1).

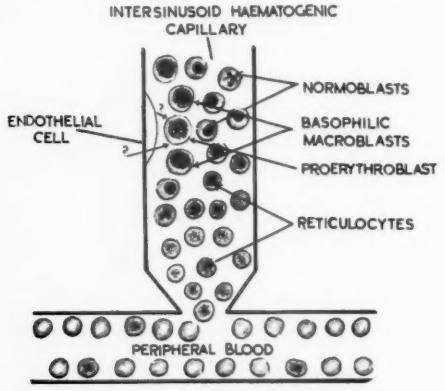

FIG. 1.—Modification of Witts' schematic diagram of the erythron. The scheme shows the erythrocytes entering the peripheral circulation only in the reticulated stage; megaloblasts are not considered as normal stage in the development of the erythron and therefore replaced by proerythroblasts and basophilic macroblasts; the ?-signs indicate that the origin of erythroblasts either from endothelial cells or from extravascular reticulum cells is undecided.

2. The enumeration of reticulocytes and their maturation time

The importance of obtaining accurate reticulocyte counts in investigating haemoglobin metabolism is obvious. It is necessary therefore to make some comment on the various methods of supravital staining at present in common use. Most authors use fresh wet preparations for enumerations of reticulocytes adopting the standpoint of Seyfarth (1927) that alcohol fixation and counterstaining causes the disappearance of some of the supravitally staining substance. Heilmeyer (1931, 1935) and Trachtenberg (1932), however, emphasize that in wet preparations erythrocytes with only few granules can easily be overlooked. The method of Heilmeyer and Trachtenberg has the disadvantage that clotting frequently occurs in blood incubated with a brilliant cresyl blue solution on excavated paraffin blocks and interferes with the supravital staining. One of us (H. S. B.) has for eight years used a method which is adapted from the old procedure of Widal, Abrami and Brulé (1907) and that of Heilmeyer and Trachtenberg:

To equal parts of normal saline and 3·8 per cent. solution of sodium citrate 0·1 gm. per cent. of brilliant cresyl blue is added and the solution filtered. 0·5 c.c. of this solution is placed in a small tube and 2–3 drops of fresh blood added. Blood and solution are mixed and kept in an incubator at 37° F. for 15 to 20 minutes. The tube is then centrifuged at low speed for 1 to 2 minutes and the supernatant fluid carefully poured off. Some of the clear supernatant fluid is left in the tube in order both to facilitate the making of thin films and to avoid removing the upper layer of cells which is richest in reticulocytes, the specific gravity of reticulocytes being lower than that of other R.B.C. (Key, 1921; Stephens, 1938; Gripwall, 1938). The tube is shaken or the contents mixed with a glass rod and films made from the suspension thus obtained, the films being counterstained with Leishman's stain It has been found that a short fixation (half a minute) with the concentrated stain and dilution of the stain some twenty times prevents damage to the reticulum which in these films is dark blue or black. It is important in counting reticulocytes to remember that they may be distributed unevenly; they often occur in rather larger numbers at the edge of the slide; for this reason we recommend Schilling's ' meander ' method.

We often use another quicker method based on those described by Pappenheim (1907) and later by Hawes (1909) and Cunningham (1920): A clean slide is covered with a saturated alcoholic solution of brilliant cresyl blue. The solution is ignited and allowed to burn till the slide is covered with a uniform layer of brilliant cresyl blue. The film should not be used on the day of preparation. A drop of blood is placed on the brilliant cresyl blue film and mixed with the stain. After two minutes a small drop of the supravitally stained blood is transferred to another slide and a thin film is prepared and counterstained as in the previous method. Usually the film before counterstaining is greenish-blue in colour; occasionally, however, it is a bright purple; films of this second type give unreliable results for the reticulum, being often indistinct and polychromasia may be found. The reason for this occasional failure is unknown; all such films must be discarded and the count repeated using another cresyl blue film or the other method.

Pure methyl alcohol and eosin appear to damage the reticulum; it is for that reason that the short fixation and high dilution of the stain are so important. Films stained in this way show distinct dark blue reticulum and even the smallest granule are easily seen. It is possible also to make differential reticulocyte counts and, no mean advantage over the wet film method, it is possible to check the counts after any length of time. Counts made in counting chambers with a dry objective are unreliable and always give too low a figure. Figures

of 0·03–0·2 per cent. for normal persons as given by Friedlander and Wiedemer (1929) cannot be accepted for these technical reasons.

With the knowledge that all R.B.C. are released from the bone marrow in the reticulocyte stage it is possible to estimate the daily intake of erythrocytes and haemoglobin if it be possible to estimate the time taken by a reticulated cell to mature into an adult erythrocyte.

The first experiments indicating the possibility of determining this time were performed by Pepper (1922). The aim of these investigations was to demonstrate the survival of R.B.C. in vitro and the property of supravital staining was used as a sign of survival. Pepper was able to show that in blood kept in the ice chest the number of reticulated cells was undiminished at the end of three weeks, while in the incubator these forms disappeared within forty-eight hours. Whether this was due to maturation or death of reticulocyte was not considered. Experiments performed with the express aim of determining the 'maturation time' of reticulocytes have been published by Heath and Daland (1930), Seyfarth and Jürgens (1927), Heilmeyer and Westerhäuser (1932). In contrast to these authors Mermod and Dock (1935) failed to find a decrease in the number of reticulocytes in incubated blood and suggest that 'an apparent decrease in reticulocyte count is found when only a few seconds staining time is permitted by the dry method.' They even doubt whether the reticulocyte does mature in the circulation and suggest that these are quickly destroyed in the circulation and their delivery from the bone marrow is an undesirable event. In all these experiments as well as in those of Pepper defibrinated blood was incubated and reticulocyte counts were made at long-time intervals, e.g. by Heath and Daland at twenty-four hour intervals, by Heilmeyer and Westerhäuser at eight, twenty-four, thirty-two, forty-eight and fifty-six hours. It will be shown later that changes occur in the properties of the erythrocyte within the shortest of these times. Before it is possible to draw conclusions from such experiments as to the maturation time in life the influence of the experimental conditions themselves must be studied and experiments in vitro, if possible, checked by determination of the maturation time in vivo.

It seemed that for our experiments in vitro the medium most suitable for the 'culture' of reticulocytes would be that which had already been shown to be suitable for tissue cultures, especially culture of bone marrow cells. For obvious reasons the medium had to be liquid. Successful cultures of bone marrow cells in liquid medium have been reported by Osgood and Brownlee (1936), and more recently by Israels (1940). Both authors have observed that in their media not only does division take place but also the transformation of immature myeloid elements into mature forms.

The medium recommended by Osgood and Brownlee is the same as that described by the Geys for tissue cultures and consists of a citrated balanced salt solution to which 0·1 per cent. dextrose and 35 per cent. human cord serum has been added. The fact discovered by Carrel and Ebeling (1921, 1922), that when vertebrate tissue cells are cultivated in blood plasma the rate of multiplication varies in inverse ratio to the age of the animal from which the plasma was taken, led to the replacement of the cord serum by serum or plasma of the patient whose blood was to be 'cultured' or, if this was not available, with adult's serum of the same blood group. This precaution seems to be justified although it is not known whether the same factor applies for maturation as for multiplication of cells. In the first experiments it was found that the citrated balanced salt solution did not prevent clotting for a sufficiently long time. An addition of a 3·8 per cent. solution of sodium citrate was therefore used in all later experiments.

To two parts of an autoclaved solution of the following composition

$Na_2C_6H_5O_7.H_2O$	5·0	gm.
NaCl	6·8	,,
$NaHCO_3$	0·23	,,
KCl	0·37	,,
$CaCl_2$ (anhydrous)	0·17	,,
$MgCl_2.6H_2O$	0·21	,,
$Na_2HPO_4.2H_2O$	0·15	,,
KH_2PO_4	0·03	,,
$MgSO_4.7H_2O$	0·07	,,
Dextrose	1·0	,,

per litre, one part of a sterile 3·8 per cent. solution of sodium citrate is added and this is mixed with 35 per cent. sterile human serum or plasma. A number of small sterile tubes provided with corks are filled with 0·5 c.c. of this medium and two drops of the blood to be examined are added under sterile precautions. Two drops of a saturated solution of brilliant cresyl blue in normal saline are added immediately to one tube and all are placed in an incubator. After fifteen to twenty minutes the tube with brilliant cresyl blue is centrifuged for one to two minutes at low speed, the supernatant fluid pipetted or poured off and films prepared and counterstained in the way described above. At time intervals of two hours brilliant cresyl blue solution is added to two other tubes, the tubes incubated for a further fifteen to twenty minutes and films prepared and stained. The remaining tubes are gently shaken every two hours. Two to twenty thousand red cells are counted depending on the number of reticulocytes present. Usually four films are made, two from each of the duplicate tubes, at each time interval. At the conclusion of the experiment the numbers of reticulocytes are plotted against the time of incubation.

The results of this method have been compared with those obtained by incubation of heparinized whole blood, whole blood in paraffined tubes, citrated whole blood and with suspensions of red cells in Osgood's medium without serum, with and without glucose and in Simmel's (1925) solution (NaCl 8·2, KCl 0·2, $MgCl_2$ 0·2, $CaCl_2$ 0·2, NaH_2PO_4 0·1, $NaHCO_3$ 0·05 gm. per litre H_2O) to which one-fifth part of 3·8 per cent. sodium citrate solution had

been added. Heparin blood and to a less degree citrated whole blood were found to be liable to haemolysis whereas whole blood in paraffined tubes remained unclotted for only a few hours; these methods were therefore discarded. In all other media the results were practically identical whether they contained serum or glucose or not. For most of the experiments which are to be reported in this paper therefore either Osgood's balanced salt solution with additional sodium citrate but without glucose and serum or citrated Simmel's solution have been used. Spectroscopic examination showed that under the conditions described there was no haemolysis for forty-eight to seventy-two hours. The importance of the dilution factor in preserving the vitality of R.B.C. has been recognized in all recent work on blood storage.

In conformity with the results of Heath and Daland and of Heilmeyer and Westerhäuser not only has a gradual decrease of the number of reticulocytes been observed but also a loosening of the reticulum, a diminution in the amount of supravitally staining substance and the replacement of filaments by granules. This is illustrated by fig. 2 and 3 which show the blood of a rabbit and a guinea-pig both poisoned with phenylhydrazine, before and after five, seven and eight hours incubation in Osgood's medium respectively.

These gradual changes in the morphology of the reticular substance, together with Heilmeyer and Westerhäuser's findings of high oxygen consumption for several hours, afford sufficient proof that a vital process and not haemolysis of reticulocytes is the cause of their disappearance. No trace of haemolysis was demonstrable by spectroscopic examination at a time when the number of reticulocytes was considerably decreased or even when they had disappeared completely. This observation and the technique of supravital staining show that the objections of Mermod and Dock are not justified. The numerical decrease of reticulocytes is illustrated by charts I to III, VI and VII in which the time in hours is plotted against the number of reticulocytes expressed as percentage of the original figure.

The points follow a straight line for four to six hours (in few instances for eight hours), then the velocity of disappearance of reticulocytes decreases considerably and the slope finally becomes a gentle one or the curve may even take a horizontal course. On the interpretation of this curve depends the final calculation of the maturation time. Heath and Daland who counted reticulocytes in defibrinated blood only every twenty-four hours describe a somewhat similar 'maturation curve' and say 'if this curve is plotted with the logarithms of the reticulocyte percentage as ordinates, the result is a straight line. This type of curve is characteristic of senescence phenomena (Robertson).' This interpretation is, however, erroneous for two reasons.

First, the type of chemical reaction described by Robertson (1923) as characteristic for multiplication, growth and senescence through the whole living world is that of a reversible autocatalysed reaction which is defined by the formula $\frac{dx}{dx} = kx(a-x)$ which on integration gives finally the formula $\log \frac{x}{a-x} = ka(t-t_1)$ and is graphically represented by a S-shaped curve, whereas the curve reproduced by Heath and Daland corresponds to the formula of monomolecular reactions $\frac{dx}{dt} = k(a-x)$ which on integration gives $\log \frac{a}{a-x} = kt$. There is indeed no reason to believe that the maturation of reticulocytes, which are not nucleated cells, should follow the first formula for it has been shown by Robertson that 'it is the nucleus, therefore, that we must look for, alike for the source of the accelerative agent in cellular multiplication, and the source of autocatalytic time-relations, which distinguish all types of growth.'

Secondly, a curve with the time as abscissa and the percentage of reticulocytes as ordinates does not represent the real kinetics of maturation. For such

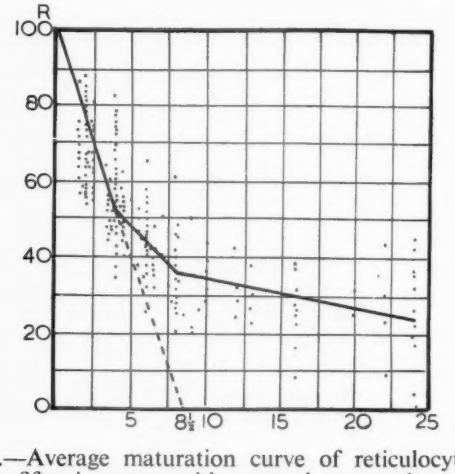

CHART I.—Average maturation curve of reticulocytes from fifty-six cases with normal or moderately increased numbers of reticulocytes. Horizontal axis—time in hours; vertical axis—reticulocytes in per cent. of original figure.

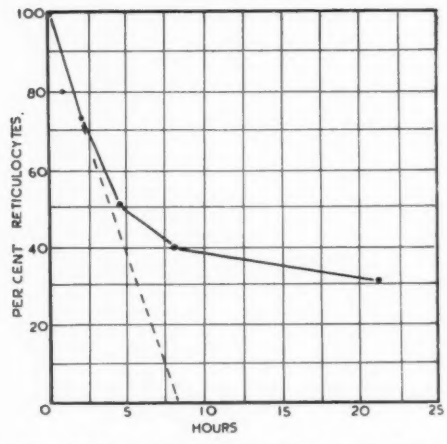

CHART II.—Maturation of reticulocytes of a normal child. Horizontal axis—time in hours; vertical axis—reticulocytes (R) in per cent. of the original figure. Extrapolation of the initial slope to R = O.

an investigation the total mass of supravitally staining substance should be plotted against time. We have, however, no means of determining the amount of reticular substance. What is determined in maturation experiments is the last stage of the reaction, the disappearance of a small, just discernible amount of reticular substance. Therefore the velocity of the reaction is estimated in a stage when (x) approaches (dx). If various films of a maturation experiment are compared, especially in cases with numerous young reticulocytes, it can easily be seen that the amount of reticular substance which disappears in the transformation of the youngest reticulocytes into cells with a loose and scanty reticulum is several times in excess of that represented by the maturation of the older reticulocytes into ordinary R.B.C. The real velocity of maturation is therefore a decreasing one even when the ' maturation curve ' is a straight line. A better insight into the real velocity of maturation can be expected if the reticulocytes are divided into several groups according to the amount of reticular substance and the velocity of disappearance determined for each group separately. Such classifications of reticulocytes have been attempted by Rosin and Biebergeill (1904), Demel (1907), Lee, Minot and Vincent (1916), Seyfarth and Jürgens (1927), Moldawsky (1928), Gawrilow (1929), Heilmeyer (1931, 1935) and Trachtenberg (1932), Eaton and Damren (1930). Heath and Daland have adopted in their studies the classification of Gawrilow into reticulocytes with large amounts of reticulum, with medium and with small amounts. Heilmeyer and Trachtenberg distinguish five groups: O = nucleated red cells, I = ball-shaped reticulum, II = network forms, III = incomplete network, IV = granular forms. In the present experiments an attempt was made to use the classification of Heilmeyer and Trachtenberg but it was found impossible to get reliable results, the transition between the various groups being ill-defined. Only if groups II to IV were combined and separated from group I was a fairly accurate differentiation possible. Chart III shows the results of such differential counts in a maturation experiment with reticulocytes of a rabbit after phenylhydrazine poisoning and curves obtained in a similar way from figures of Heath and Daland, and Heilmeyer and Westerhäuser.

The curves show that the slope is somewhat steeper for the younger forms. As has already been pointed out even when the rate of disappearance of two such groups is the same the actual ' velocity of maturation ' is higher for the forms with the large amount of reticulum, the real speed of disappearance of reticulum (not to be confused with the disappearance of reticulocytes) is therefore to be represented by a curve of diminishing slope. The maturation curves shown here have at first sight such a shape. It is of first importance to realize that the resemblance between these curves and that deduced for the rate of disappearance of reticular substance is only superficial. In the latter case the slope represents the relation between the mass of reticular substance in a single erythrocyte and the time without relation to the total number of such cells present; the present curves, however, are obtained by determining only the end point of the process and for the first part of their course follow a straight line, a rapid diminution of the slope occurring after some hours. The decreasing velocity of the chemical reaction underlying the maturation of reticulocytes therefore cannot explain the shape of curves representing the decreasing number of reticulocytes in incubated blood.

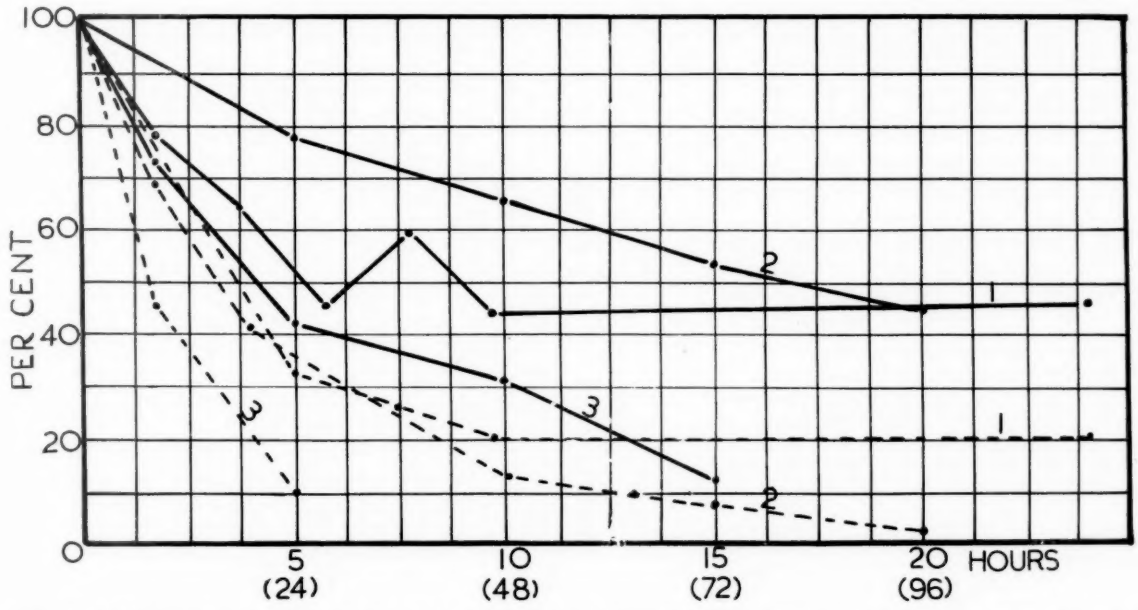

CHART III.—Maturation of reticulocytes with regard to their immaiurity. Horizontal axis—time in hours; vertical axis—reticulocytes in per cent. of original figure. ——— Drop in the total number. – – – Drop in the number of group I reticulocytes. 1. Pair of curves: Maturation of rabbit's No. 3 reticulocytes in vivo. 2. Pair of curves : constructed from figures of Heath and Daland. 3. Pair of curves: constructed from figures of Heilmeyer and Westerhäuser. The figures in brackets correspond to curves 2 and 3.

It is possible to explain in two ways why the reticulocytes disappear so rapidly in the early hours of incubation while they later disappear slowly or remain present in a constant percentage.

(a) It is conceivable that the maturation of reticulocytes results in a formation of substances which diffuse into the medium and inhibit the maturation of the remaining reticulocytes. This possibility is suggested by experiments of Eijkman who has shown that substances are developed in bacterial cultures which inhibit their further multiplication and by those of one of us (H. S. B., 1912) who has demonstrated the production of substances having a comparable inhibiting action during the swelling and germination of seeds of Phaseolus and Atriplex. In order to find out whether this possibility applies to the maturation of reticulocytes two series of tubes containing blood in citrated Simmel's solution were incubated. One series was used for a maturation experiment in the usual way while in the other the medium was pipetted off and replaced every hour. The results of two such experiments, one on a guinea pig with phenylhydrazine poisoning, the other on a baby with erythronoclastic anaemia are shown in table 3.

TABLE 3

MATURATION OF RETICULOCYTES IN A CASE OF ERYTHRONOCLASTIC ANAEMIA AND IN A GUINEA PIG WITH PHENYLHYDRAZINE ANAEMIA. SERIES I REPRESENTS A USUAL MATURATION EXPERIMENT; IN SERIES II THE DILUTING FLUID HAS BEEN REPLACED HOURLY

Hours of incubation	Number of reticulocytes			
	Baby H.		Guinea pig No. 22	
	I	II	I	II
0	10·2(100)	—	38·6(100)	—
1	—	—	33·4(86·5)	—
2	7·2(71)	7·4(72·5)	26·2(68·0)	27(70)
4	3·2(31·4)	2·8(27·5)	19·0(49·5)	20·5(53)
5	2·5(24·4)	2·6(25·5)	—	—
6	1·5(14·7)	1·2(11·7)	15·4(40)	13·2(34·2)
8	0·9(8·8)	0·8(7·85)	12·2(31·4)	11·9(30·8)
24	—	—	—	—

(Figures in brackets represent percentages)

The maturation curve in the two series was practically identical and the assumption of inhibiting substances diffusing into the medium must therefore be abandoned.

(b) The retarded maturation might be due to changes occurring in vitro in the erythrocytes. Meulengracht (1922) has demonstrated an increase in the fragility of R.B.C. kept in the incubator or at room temperature. In recent years a thorough study of the changes in the sedimentation rate and corresponding transformation of normal erythrocytes into spherocytes has been performed by Bergenhem and Fåhraeus (1936, 1939). Extensive investigations on changes in stored red blood cells have recently been published by Maizels and Whittaker (1940), Bushby, Kekwick, Marriott and Whitby (1940), Maizels and Paterson (1940), Aylward, Mainwaring and Wilkinson (1940), Dubash, Clegg and Vaughan (1940). These investigations have shown that a decrease in the sedimentation rate occurs and spherocytosis develops in incubated blood and that stored R.B.C. show an increase in the mean corpuscular volume, a decrease in the content of potassium and inorganic phosphorus and an increased fragility in hypotonic NaCl solutions. The investigations on stored blood, however, were made on blood stored at low temperatures and twenty-four hours was the shortest interval. Table 4 shows the results of potassium determinations in plasma of citrated blood incubated for four, six and eight hours. It shows that there is no change in the plasma potassium in the first four hours, while later a considerable diffusion occurs from the cells into the plasma.

TABLE 4

CHANGES IN THE PLASMA POTASSIUM ON INCUBATION OF CITRATED BLOOD

Case	Plasma potassium after hours of incubation			
	0	4	6	8
I	8	10	26	28
II	9	10	14	24
III	15	15	17	31

Changes in the fragility have been studied in blood incubated with the addition of one-fifth of 3·8 per cent. sodium citrate solution and in blood diluted five times with citrated Simmel's solution. Quantitative estimations of fragility were made by a method similar to that of Waugh and Asherman (1938): 0·02 c.c. of blood were added to 12 c.c. of distilled water and 12 c.c. of nine solutions of NaCl in concentrations 0·30, 0·35, . . . 0·70 per cent. respectively, in centrifuge tubes. The tubes were gently shaken and allowed to stand at room temperature for one hour. They were then centrifuged and the supernatant fluid pipetted off into tubes of the macroapparatus of Evelyn's photoelectric stepphotometer. The amount of haemoglobin was then determined in each, using the filter 540 and the percentage haemolysis calculated, taking the amount in the tube with distilled water as 100 per cent. Theoretically the enumeration of non-haemolysed erythrocytes as in Simmel's (1925) method or that of Whitby and Hynes (1935) is more correct than the determination of haemoglobin in the solution as in this method or those of Bauer and Aschner (1919) or Creed. The red cell population consists of cells with varied volume and varied haemoglobin content. The proportion of small and large cells is not necessarily identical in those which undergo haemolysis and those which do not. This factor seems, however,

to be not significant as Momigliano-Levi and Bairati (1935a) failed to find any relation between the diameter of R.B.C. and fragility and parallel determinations with Simmel's method and that described above have not shown significant differences. Haemoglobin determinations were chosen as being the more accurate and less laborious method. For graphic representation the curve of May (1914) and Momigliano-Levi and Bairati (1935a and b) has been chosen which is a distribution curve of an R.B.C. population divided in classes of various fragility, each class comprising the cells escaping haemolysis at the given NaCl-concentration and being haemolysed by the next lower concentration. Such a distribution curve appears to be more instructive than the 'integral' curves of May (1914), Whitby and Hynes (1935), Vaughan (1937), or the hatched columns in Simmel's method. Charts IV and V show the changes in fragility in citrated blood and in blood diluted five times with citrated Simmel's solution. A distinct increase in the fragility is seen from the shift of the curve to the right side after four hours incubation in the first case and after eight hours in the second.

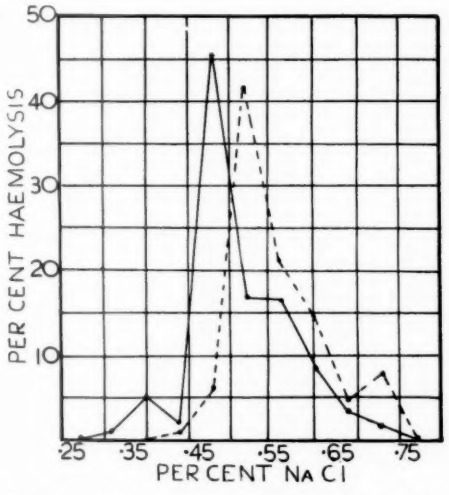

CHART IV.—Fragility curve of normal R.B.C. ——— before incubation, – – – – – – after four hours incubation. Horizontal axis: concentration of hypotonic NaCl-solutions; vertical axis per cent. haemolysis corresponding to the class interval indicated on the horizontal axis.

Further changes in incubated R.B.C. are revealed by morphological examination. After four to eight hours' incubation a number of erythrocytes show crenation when examined in wet preparations and changes in staining properties in fixed films. Especially often are such changes found if supravital staining precedes the Leishman-stain and they are more obvious if supravital staining is performed on a dry brilliant cresyl blue film than in isotonic solution. The red cells are often paler or irregularly stained, sometimes greyish in colour. Some such changes can be detected in fig. 2 and 3. If at the same time samples of the incubated blood are examined in films stained with Leishman's stain or with Pappenheim's method but without previous supravital staining the R.B.C. do not as a rule show any morphological changes. Hirschfeld and Hittmair (1925) have shown that brilliant cresyl blue damages the R.B.C. Incubated R.B.C. are obviously more susceptible to this injury and after a short incubation period this higher susceptibility to damage by supravital staining is usually the only morphological evidence that the cells are not perfectly normal.

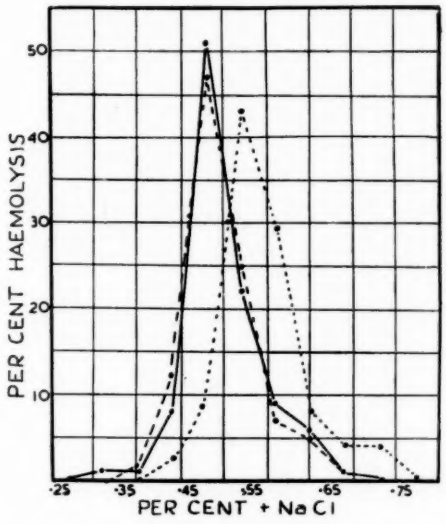

CHART V.—Changes in fragility of incubated normal R.B.C. Diluted five times with citrated Simmel's solution. Horizontal axis : concentration of the hypotonic NaCl-solutions; vertical axis per cent. haemolysis corresponding to the class interval indicated on the horizontal axis. ——— before incubation, – – – – – – after four-hours incubation, ... after eight-hours incubation.

That such damaged erythrocytes are still capable of performing their function is clearly seen from the experiments of Bushby, Kekwick, Marriott and Whitby (1940) which show that a considerable number of stored R.B.C. with an increased fragility survive after transfusion for several days in the recipient's circulation. They are depressed in their vitality, necrobiotic to some extent, but not necrotic. The significance of these changes becomes evident if two important findings are born in mind. Maizels and Paterson (1940) found that stored R.B.C. which have lost some of their potassium ions and gained some sodium become readjusted to their normal composition if transfused. As the figures are of such magnitude that the readjustment cannot be explained by a simple physical process they assume a vital action of the body tissues especially of the spleen, which organ Stephens (1939) suggests may have the function of prolonging the life of R.B.C. Bushby, Kekwick, Marriott and Whitby (1940) found a close correlation between the fragility of stored erythrocytes and the percentage survival two and three days after transfusion. The changes occurring in incubated blood must therefore be considered as indicating a decrease in the vitality of R.B.C. It is therefore concluded that in blood incubated for only a few hours the erythrocytes can be considered as perfectly normal, but after four to eight hours, even under optimal conditions, the

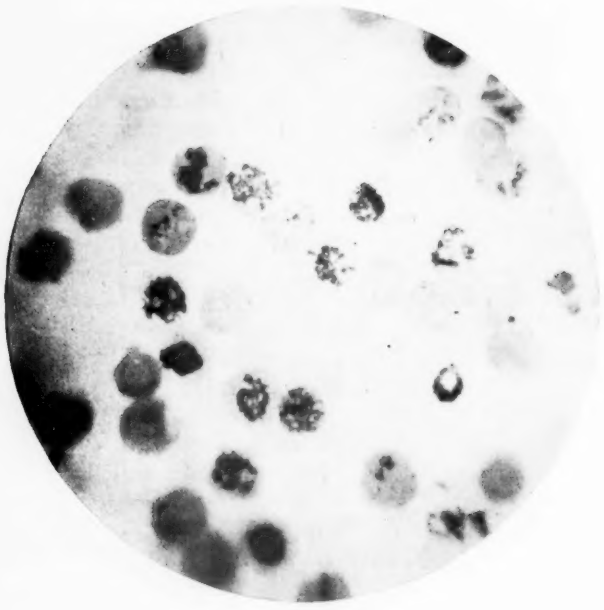

FIG. 2a.

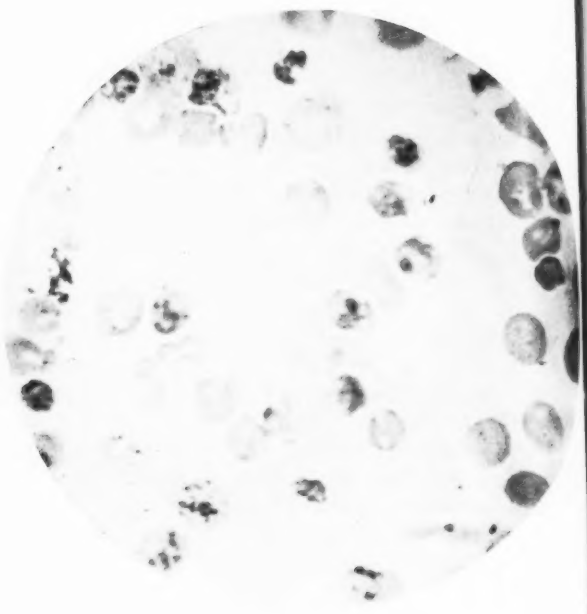

FIG. 2b.

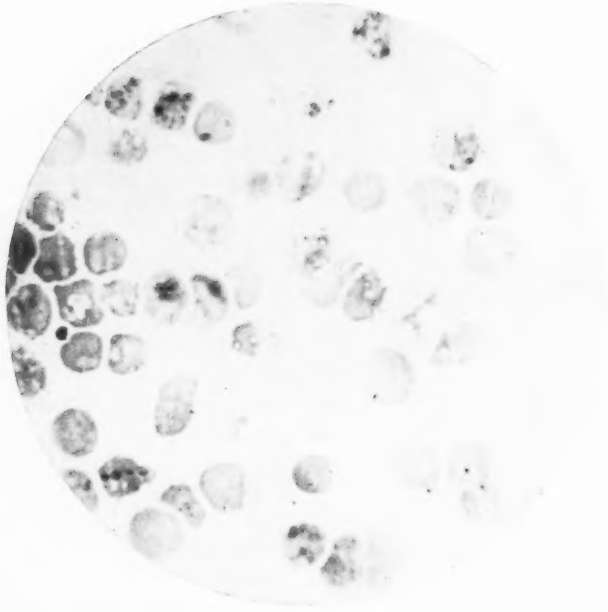

FIG. 2c.

FIG. 2.—Blood films of rabbit 9 supravitally stained and counterstained before incubation (a), after five (b) and after seven hours' (c) incubation.

metabolic processes lead to a decrease of vitality and the initiation of a necrobiotic process. At this time a considerable retardation is observed in the maturation curve. In the body there is always opportunity for correction of adverse metabolic changes; this retardation is therefore to be considered as an artificial phenomenon due to the unnatural conditions, and the first few hours only of a maturation experiment can be considered as an imitation of the vital process. **The time necessary for the youngest reticulocytes in the blood to be transformed into mature erythrocytes is therefore not given by the time when all or even most of the reticulocytes have disappeared in vitro; it can only be obtained by extrapolation of the first straight and steep slope to ordinate zero.**

If this method is applied to the average maturation curve in chart I a maturation time of eight and a half hours is obtained. This curve, however, includes many cases with increased numbers of reticulocytes, some of them younger than those found in normal blood. In normal cases (they are for technical reasons too few to calculate an average) figures between six and eight hours are usually found.

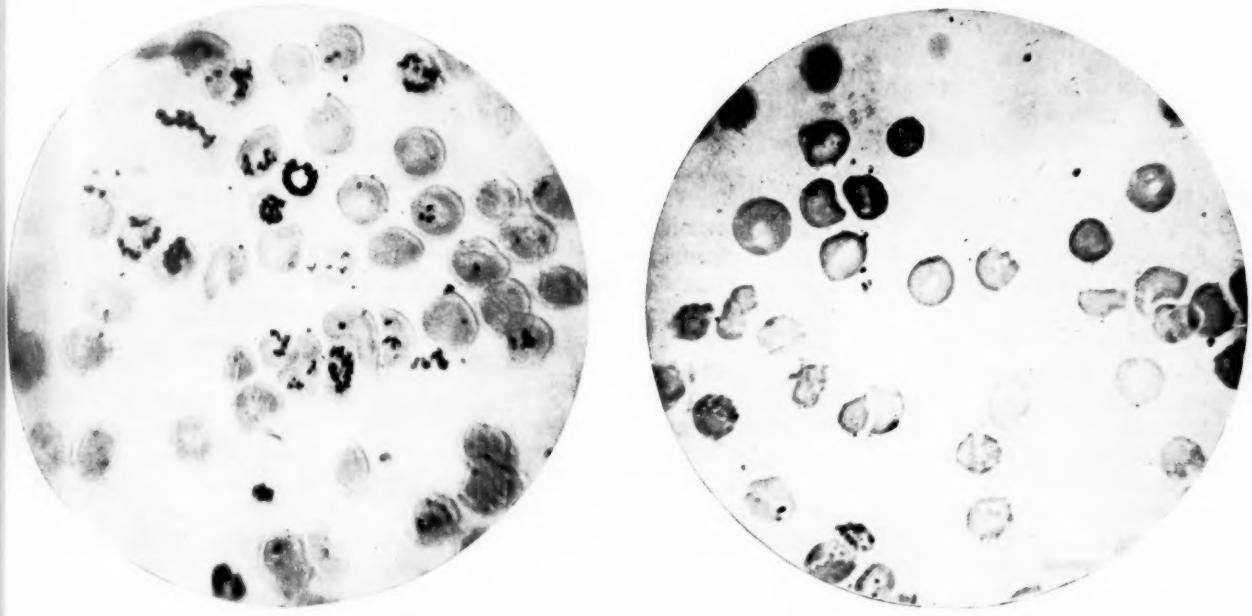

FIG. 3a. FIG. 3b.

FIG. 3.—Blood films of guinea pig No. 22 supravitally stained and counterstained before incubation (a) and after eight hours' incubation (b).

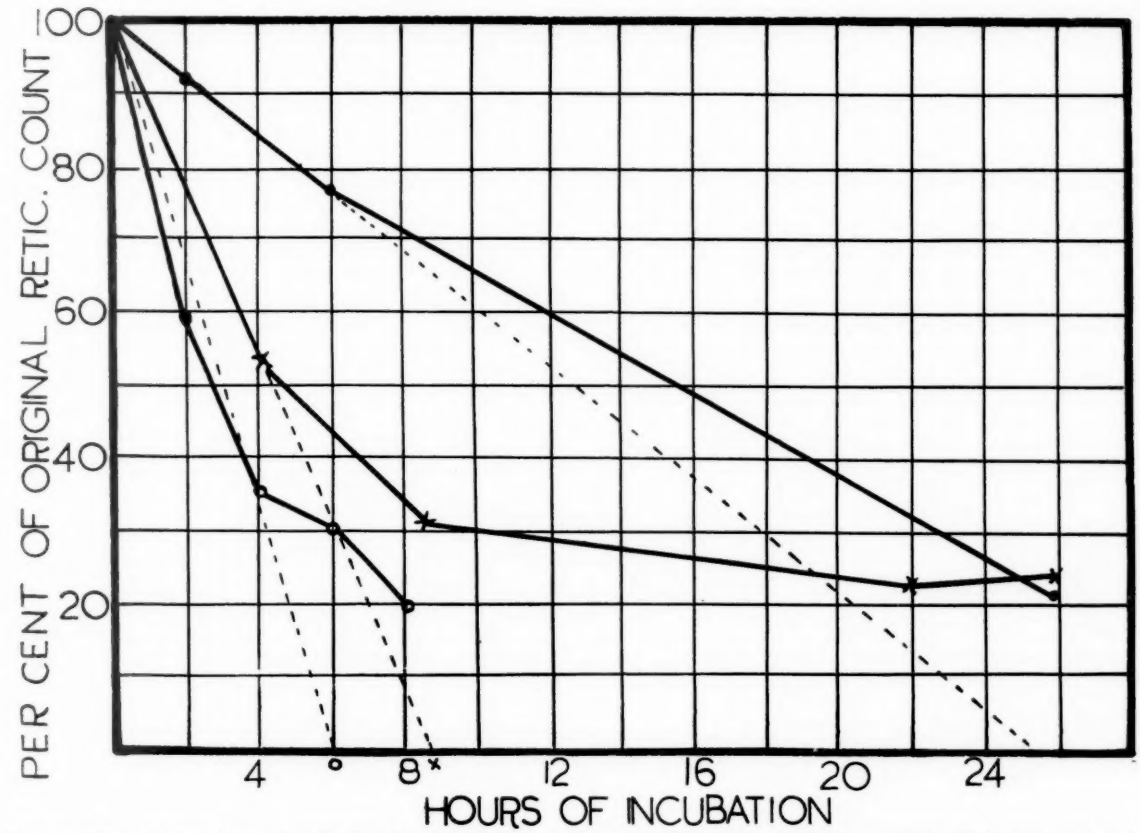

CHART. VI.—Maturation of reticulocytes in vitro. x——x case of erythronoclastic anaemia (7·6 per cent. reticulocytes), o———o M.P., acholuric jaundice (12·8 per cent. reticulocytes), .———. S.P., acholuric jaundice (14·2 per cent. reticulocytes).

The determination of the maturation time in every case investigated for haemoglobin metabolism is a somewhat laborious procedure. It would certainly be a convenience if it were possible to correlate the maturation time with the total reticulocyte count or with the differential count of reticulocytes. There can be no doubt that the maturation time is lengthened by the appearance of many young forms of reticulocytes and the longest maturation times have been found in cases of icterus gravis neonatorum and of acholuric jaundice in which considerable numbers of the youngest forms of reticulocytes were present. There has, however, not been a numerical correlation; in some cases with high reticulocytosis and many young forms, normal maturation times have at times been found. This lack of any mathematical relation between the number of reticulocytes and their maturation time is shown in chart VI. Two of the cases represented in this chart with 12·8 and 14·2 per cent. reticulocytes were cases of acholuric jaundice; it may be significant that the one with a maturation time of six hours was perfectly compensated whilst the other with a maturation time of twenty-five hours was anaemic and had a history of haemolytic crises. Chart VII shows an individual maturation curve of a case of icterus gravis neonatorum with an exceptionally long maturation time of forty hours.

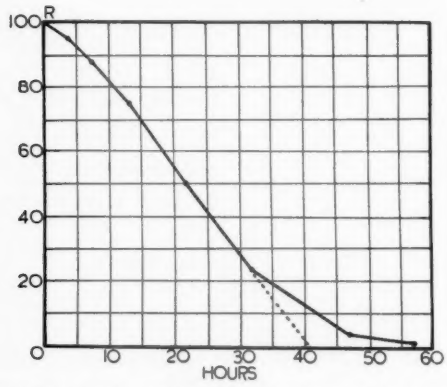

CHART VII.—Maturation of reticulocytes in vitro in a case of erythronoclastic anaemia of the newborn.

These findings suggest that besides the differences due to the age of reticulocytes there are also some intrinsic differences in the speed of maturation.

The arguments discussed above suggest that it may be correct to calculate the maturation time by extrapolation of the initial slope. The crucial experiment, however, the estimation of the maturation time in vivo, was attempted by counting the reticulocytes after transfusion of blood showing a high reticulocytosis into normals. This operation was only once carried out in man, when, in the course of an experiment of a quite different character, blood from a case of acholuric jaundice (M.P.—Chart VI) was transfused into an infant with hydrocephalus and meningocele. The reticulocyte count of the infant was raised by 5·4 per cent.; after six hours it had nearly and after eight hours completely fallen to the pre-transfusion level. This result agreed well with that found in vitro (six hours, M.P., chart VI). Counts for the maturation in vitro were in this instance checked three times, two stained films and two wet preparations being examined at each time interval.

Similar experiments were made on rabbits rendered anaemic by subcutaneous injections of phenylhydrazinc hydrochloride; this poison was chosen as it produces the highest reticulocyte responses. Various standard doses have been recommended: it was found inadvisable to follow any set scheme in producing phenylhydrazine anaemia; not only are there differences between various animals (e.g. guinea pigs were more resistant than rabbits) but also individual differences in the same species. These observations are in agreement with findings of Evensen (1938) who noticed individual differences in the resistance to the drug among human beings and also differences in the various age groups. In later experiments a start was made with 0·05 gm. phenylhydrazine hydrochloride in adult and 0·02 gm. in young rabbits and adjusted the subsequent doses and time intervals to the degree of the resulting anaemia. A number of animals were rendered anaemic in this way and three were used for transfusion experiments.

Rabbit 1. Weight 2270 gm. Haemoglobin 12·7–13·5 gm. per cent. R.B.C. 6·4–6·96 millions per c.c. Reticulocytes 1·2–3·3 per cent. On Jan. 24, 1940, 0·25 gm. phenylhydrazine hydrochloride given. Further injections: Feb. 1, 1940, 0·04 gm.; Feb. 6, 0·06 gm.; Feb. 9, 0·05 gm.; Feb. 13, 0·05 gm. On Feb. 15 the reticulocyte count was 70 per cent. On the next day when the haemoglobin was 8 gm. per cent., the red cell count 3·83 millions and the reticulocytes 55·7 per cent., 16 c.c. of blood were removed by heart puncture and immediately injected into the marginal vein of a young rabbit (No. 3) which had 7·45 gm. per cent. haemoglobin, 4·43 millions R.B.C. and 4 per cent. reticulocytes before the transfusion. Twenty-five minutes after the injection the haemoglobin was 10·2 gm. per cent. and the reticulocytes 15·6 per cent. At two-hour intervals blood was taken for haemoglobin determinations and reticulocyte counts. Two hours after the transfusion blood was taken from the recipient and the maturation time estimated in vitro; this experiment was repeated on the next day. A corresponding determination with the blood of the donor had been made on the previous day. The results of this experiment are represented in chart VIII where the solid line corresponds to the reticulocyte counts of rabbit No. 3 after transfusion (maturation in vivo), the dotted line to the counts on the blood taken from this rabbit two hours after transfusion and incubated in Osgood's solution, the dashed line to the maturation in vitro of the donor rabbit's (No. 1) reticulocytes.

Rabbit No. 9. Body weight 2100 gm. Haemoglobin 12·14 gm. per cent. Reticulocytes 1·2 per cent. On June 24, 1940, subcutaneous injection of 0·04 gm. phenylhydrazine hydrochloride in 2 c.c. of normal saline. Further injections on July 1, 1940, 0·03 gm. and on July 3, 0·03 gm. On July 5, haemoglobin 4·97 gm. per cent., reticulocytes 47·3 per

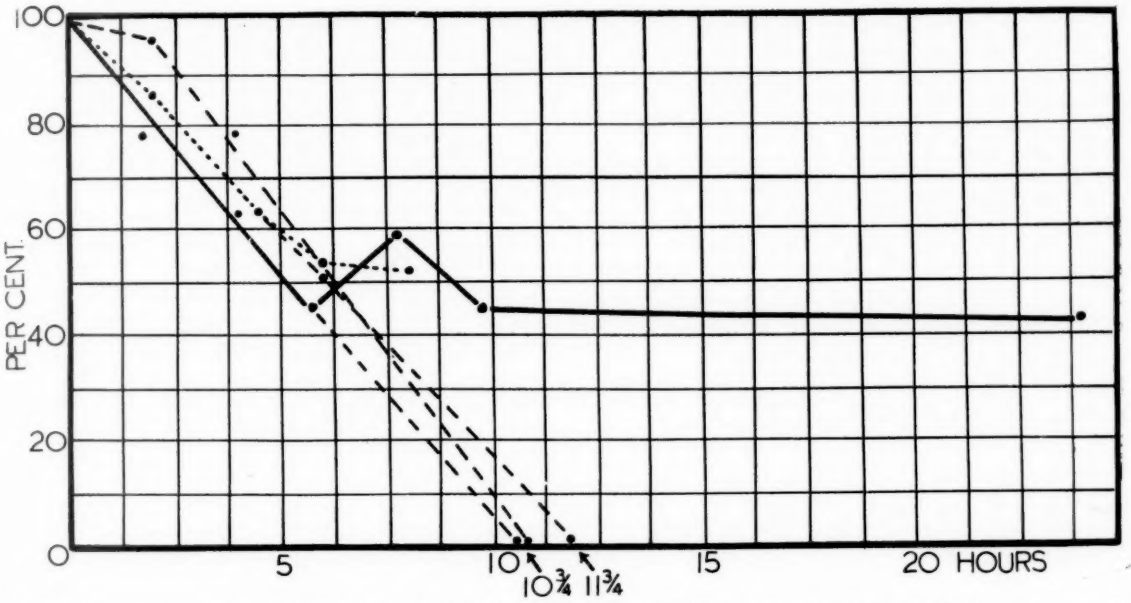

CHART VIII.———Disappearance in vivo of reticulocytes of rabbit No. 1 transfused to rabbit No. 3. – – – – Disappearance of rabbit's No. 1 reticulocytes in vitro. ..... Disappearance in vitro of rabbit's No. 3 reticulocytes taken after transfusion. Horizontal axis—time in hours; vertical axis—reticulocytes in per cent. of original figure.

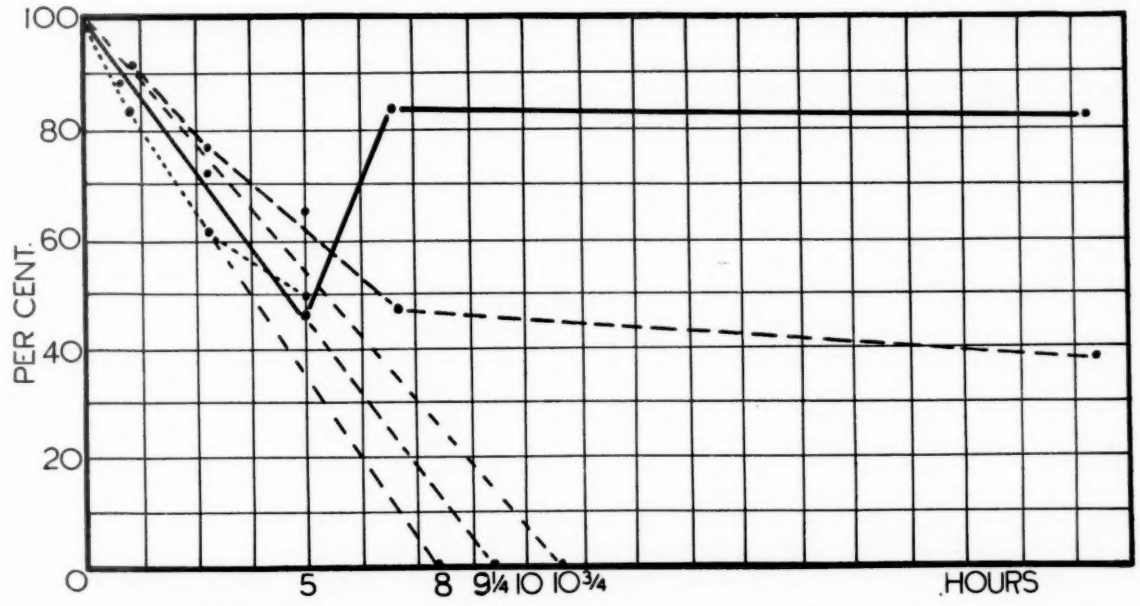

CHART IX.——— Disappearance in vivo of reticulocytes transfused from rabbit No. 9 to rabbit No. 10. – – – – Disappearance of rabbit's No. 9 reticulocytes in vitro. ..... Disappearance in vitro of rabbit's No. 10 reticulocytes taken after transfusion. Horizontal axis—time in hours; vertical axis—reticulocytes in per cent. of original figure.

cent. On this day 12 c.c. of blood were withdrawn by heart puncture and immediately injected into the marginal vein of rabbit No. 10 which before transfusion had 9·93 gm. per cent. haemoglobin and 3·5 per cent. reticulocytes. Fifteen minutes after transfusion the haemoglobin level was 11·3 gm. per cent. and the reticulocyte count 10·03 per cent. Successive reticulocyte counts and parallel determinations of the maturation time in vitro have been performed as in the first experiment. The results are seen in chart IX.

In both experiments there was a linear drop in the number of reticulocytes for five to six hours. But afterwards, following a short rise, the level remained unchanged for more than twenty-four hours. Whilst the initial drop was expected and was in agreement with the experiment on a human being the secondary rise and the high number of reticulocytes remaining constant for a long time came as a surprise. It is difficult to explain this observation. Perhaps the most reasonable explanation is to assume that with

the transfused blood traces of phenylhydrazine or more probably some disintegration products of haemoglobin, suggested by the brownish colour of the blood in phenylhydrazine poisoning, were injected, which acted as a stimulus to the recipient's bone marrow and caused a reticulocyte response which concealed the final disappearance of transfused reticulocytes. If, however, the initial slope is extrapolated to the ordinate corresponding to the pre-transfusion level a close agreement with the results of the maturation experiments in vitro is obtained; thus for the reticulocytes of rabbit 1 a maturation time of $10\frac{1}{2}$ hours was found in vivo and $11\frac{1}{2}$ hours in vitro. For those of rabbit 9 the corresponding figures are $9\frac{3}{4}$ and $11\frac{1}{2}$ hours.

The supposed stimulus appears to have been stronger and more rapid in action in a third experiment where the number of reticulocytes two hours after transfusion was higher than immediately after transfusion and remained at this level for more than twenty-four hours.

From the experiments on one human being and two rabbits it may be concluded that the **determination of the maturation time of reticulocytes in vitro by the method described above with extrapolation of the linear slope to ordinate zero gives figures closely corresponding to those obtained under vital conditions.** Further experimental proof for these conclusions will be given in the next paper (part XIII).

The difference between these results and those of Heath and Daland, and Heilmeyer and Westerhäuser is probably due to the fact that these authors used undiluted defibrinated blood, that the first authors made their counts at twenty-four hour intervals only and the latter made their first count after eight hours; moreover both considered as the maturation time the time at which all or nearly all reticulocytes had disappeared.

3. Discussion of the indirect determination of the reticulocyte's maturation time

Finally the results of the present experiments must be compared with those obtained by authors who tried to determine the maturation time of reticulated R.B.C. indirectly. Riddle (1930), from observations on the regeneration rate of R.B.C. during the liver treatment of pernicious anaemia, concluded that the reticulocytes delivered from the bone marrow develop into mature erythrocytes in about two days. Riddle's application of Robertson's formula for growth and multiplication to the regeneration of erythrocytes has been already mentioned above. In one case analysed in detail Riddle has shown that if such a formula is applied to the delivery of reticulocytes and from the calculated numbers of reticulocytes at a given time (t) the number for the time two days before (t−2) be subtracted the resulting curve is almost identical with the curve of reticulocyte concentrations really observed. The conclusion, however, that the maturation time was two days in this case is only valid if all the R.B.C. produced during this time and those previously present remain intact in the circulation, in other words, if cell destruction is negligible. Such a conception would be in accordance with the teaching of Whipple and Minot at the time when the ' maturation blockade ' was considered to be the one essential factor in pernicious anaemia, and earlier anatomical and biochemical findings indicating a haemolytic process in this disease were disregarded. There is no reason to discuss in detail the theory that the megaloblast is a stage in the normal development of the erythrocyte here, but it must be mentioned that in late years a number of important investigations have been published by Rhoads, Dobriner and their collaborators which prove the increased destruction and production of erythrocytes in pernicious anaemia. The theory of Whipple (1917a and b, 1922, 1928) that bilirubin and stercobilin are derived only in part from the haemoglobin of R.B.C. and in part from myohaemoglobin and a hypothetical ' pigment complex ' formed outside the R.B.C. has been strongly criticized by Heilmeyer (1931a) and is rejected by most authors who have worked on pigment excretion (cf. Watson, 1938), nor indeed does it explain the increased excretion of coproporphyrin I demonstrated by Dobriner and Rhoads (1938) which according to Fischer's dualism of porphyrins, must be regarded as a by-product of haemoglobin formation. A ' haemoglobinolysis ' without ' haemocytolysis ' as assumed by Jedlicka (1930) is completely hypothetical and contradicts the well-founded ' all or none law ' of haemolysis (Ponder, 1934; Saslow, 1929). The only possible explanation of increased pigment excretion without destruction of circulating R.B.C. is therefore erythrophagocytosis within the bone marrow. This has indeed been described by Peabody and Broun (1925) and by Doan (1926), who has studied erythrophagocytosis in pernicious anaemia by the supravital technique. Doan found in the bone marrow an increase in the number of clasmatocytes, a term restricted by this author to phagocytic cells of endothelial origin. These cells take up not only mature red cells but also nucleated R.B.C. in cases showing only an occasional nucleated R.B.C. in the peripheral circulation. In the bone marrow of a twelve-year-old boy who died from typical pernicious anaemia complicated by septicaemia the findings of these authors have been confirmed. The erythrophagocytosis was not conspicuous, however, and in a bone marrow overcrowded with megaloblasts only occasionally was a nucleated red cell found in a phagocytic cell. More erythrophagocytes than in the bone marrow were seen in the spleen and in the lymph nodes. We agree therefore with Watson (1938) that the destruction of R.B.C. is not significantly increased by phagocytosis of cells not yet delivered into the circulation.

The intensity of red cell destruction during the reticulocyte response in pernicious anaemia is especially evident in the observations of Schiodt (1938b). In one of his cases this author found on the third day of liver treatment one million R.B.C. per c.mm. with 3 per cent. reticulocytes; on the sixth day 33 per cent. reticulocytes and still only

one million R.B.C. The absolute figures of the reticulocytes were therefore 30,000 and 330,000 respectively. Thus in the course of three days 300,000 non-reticulated cells had disappeared. Even if the most unlikely assumption is made that during these three days none of the reticulocytes had either matured into adult R.B.C. or had been destroyed this figure would correspond to a daily destruction of 10 per cent. of the total number of erythrocytes. Figures allowing a similar calculation can be found in the paper of Goldhammer, Isaacs and Sturgis (1934). An observation reported by Ashby (1921) of a patient with pernicious anaemia belonging to group A who after two transfusions of group O blood had 930,000 R.B.C. group O and 300,000 group A cells and on the next day 930,000 R.B.C. all being group O cells is a further incontrovertible proof of an increased, in this case selective, destruction of erythrocytes in pernicious anaemia. Morawitz (1928) has reported an observation identical with that of Ashby. The inferior quality of R.B.C. in pernicious anaemia is evident from investigations of Deutsch and Wagenfeld (1931) who have shown that the normal increase of oxygen consumption by erythrocytes in presence of liver extract is absent if R.B.C. from pernicious anaemia are used for the experiment. The view of Rous (1923) therefore is as valid to-day as it was eighteen years ago, '. . . there are investigators who maintain that pernicious anaemia is not a disease of blood destruction at all. They put isolated facts or ingenious hypotheses against the mass of data which attests to havoc among the red cells.'

If the destruction factor is to be introduced in Riddle's calculation its magnitude must be estimated at least approximately. This is possible on the basis of stercobilin figures given by Eppinger (1913, 1920), Lichtenstein and Terwen (1925), Farquarson, Borsook and Goulding (1931), Heilmeyer (1932), Belongowa (1928) and Watson (1931). These figures represent only a part of the real destruction quota but they are useful if expressed as multiples of normal figures obtained by the same method. From these figures it can be calculated that patients with pernicious anaemia excrete more than five times as much stercobilin as normal persons with a normal amount of circulating haemoglobin. The same figure was obtained from the investigations of Jones (1922) who examined the duodenal contents after administration of magnesium sulphate. The average amount of bile pigment in six fractions taken at fifteen-minute intervals was 40 units (Willbur and Addis, 1914) for normal persons and 194 for pernicious anaemia. During the first stage of liver treatment, up to the peak of reticulocyte response, the pigment excretion is usually high, even higher than before treatment (Watson, 1938; Filo, 1931; Farquarson, Borsook and Goulding, 1931). In a normal person with a constant haemoglobin level the daily destruction must equal the daily production. It is therefore permissible to express the daily destruction in pernicious anaemia as a multiple of the normal reticulocyte concentration without knowledge of the maturation time for the reticulocytes. The result in Riddle's case can be demonstrated by a simple calculation. The figures for the reticulocyte concentrations determined at twelve-hour intervals were read from Riddle's graph. The sum of eighteen figures is 4,480,000. In the same period there was an increase in erythrocytes of one million or about one-fourth of the sum of reticulocyte concentrations. This means that in the absence of all destruction a maturation time of four times twelve hours or two days should be concluded, which agrees exactly with Riddle's different calculation. The result, however, becomes different if the destruction is taken in account. The normal destruction is equivalent to an average reticulocyte count of 0·8 per cent.* or, with five millions R.B.C. per c.mm. to a reticulocyte concentration of 40,000 per c.mm. This figure multiplied by five gives 200,000 reticulocytes per c.mm. as the reticulocyte concentration necessary to balance the average destruction of R.B.C. in pernicious anaemia. If this figure is subtracted from every figure included in the sum of 4,480,000 the resulting sum is 880,000, actually less than the real gain in erythrocytes; the maturation time must be therefore less than twelve hours; $\frac{0{\cdot}88}{1{\cdot}0} \times 12 = 10{\cdot}6$ hours is the figure calculated on this basis for the maturation time in Riddle's case. There has not been any opportunity of determining the maturation time in vitro during a reticulocyte response in pernicious anaemia, but in other conditions with comparable numbers of reticulated cells figures as the one calculated above have often been met.

Only twice has it been possible to determine the maturation time of reticulocytes in untreated pernicious anaemia of adults. One was a typical case with hyperchromic anaemia, megalocytosis, haematinaemia, the usual clinical findings and recovery after liver treatment. The other one had a severe hyperchromic anaemia, normal or slightly increased reticulocyte counts, an increased mean diameter of R.B.C. with an heterogeneous Price-Jones curve, considerably increased amounts of stercobilin in the stools, and urobilinuria, but did not react to liver treatment. Post-mortem findings corresponded to those of pernicious anaemia. It is supposed that this case belongs to that group of atypical pernicious anaemia which has been described by Wilkinson and Israels (1935a and b) as achrestic anaemia. These two cases were the only ones in which the maturation time was considerably below the normal range, namely two to three hours. It is certainly not permissible to draw conclusions from two cases, one being an atypical one, but with all reserve, it may be suggested that this finding may in part explain the striking discrepancy between the low reticulocyte counts in this condition and the considerable increase in the excretion of coproporphyrin I, the latter

* 0·8 per cent. has been taken in this case and not 0·7 as in other instances, the first figure being that accepted by the quoted authors (Heilmeyer, Watson).

indicating a high regeneration rate (Dobriner and Rhoads, 1938).

A remarkable attempt to determine the maturation time of reticulocytes in an indirect way has been made by Kurtz (1937). This author determined the blood volume (V), the red cell count in millions per c.mm. (E) and the reticulocyte concentration (R) in a number of rabbits. He then calculated that if L be the daily loss of R.B.C., a loss compensated by the delivery of reticulocytes, $V \times R = L \times E$ or $R/L = E/V$ and $L = VR/E$ if R represents the daily production of red cells, or in other words if the reticulocyte maturation time is twenty-four hours. The average for E/V he found to be 35,900. Next, a series of animals was bled daily, the amount of blood lost each day being a known figure (L_1). If the new reticulocyte concentration be R_1, $\frac{R_1-R}{L_1}$ is the absolute reticulocyte increase in millions per c.mm. for each c.c. of the daily loss and corresponds to R/L at the normal red cell level. But while L was an unknown figure calculated from the equations above on the assumption that the maturation time of reticulocytes was twenty-four hours, the figure L_1 is known. Kurtz found an average of 38,700 for $\frac{R_1-R}{L_1}$. As this corresponds closely to the figure 35,900 for E/V in normal rabbits it was concluded that the supposition for the equations, that the maturation time of reticulocytes is twenty-four hours, had been correct. The calculation, however, must be corrected at one point. The assumption that R_1-R represents the increase of reticulocytes necessary to balance the daily artificial loss of blood is only correct if R is equivalent to the daily destruction in both normal rabbits and in those made anaemic by repeated bleeding. This is not the case however. In table 7 of the next paper (part XIII), the blood destruction in rabbits with post-haemorrhagic anaemia is calculated on the basis of figures given by Gordon (1934) and a maturation time of ten hours. The corresponding figures calculated on the basis of a maturation time of twenty-four hours for the pre-experimental period and at the height of the reticulocyte response are given in brackets. The table shows that even on the latter basis the destruction figures are increased more than eight times. The number of reticulocytes necessary to balance the daily destruction must therefore be considerably higher in anaemic rabbits than in normal animals. If the number of reticulocytes necessary to balance the daily destruction in anaemic rabbits be called Ra, $\frac{R_1-Ra}{L_1}$ must be considerably smaller than $\frac{R_1-R}{L_1}$, in other words the number of reticulocytes required to compensate for the daily loss by bleeding (L_1) is much smaller than was assumed by Kurtz, their maturation time must be therefore much shorter.

4. Calculation of the regeneration rate of R.B.C., their life span and the daily haemoglobin intake

If the number of reticulocytes and their maturation time be known it is easy to calculate the regeneration rate of R.B.C. and haemoglobin, and if the blood volume be determined, the daily haemoglobin intake. The number of reticulocytes per cent. multiplied by twenty-four and divided by the maturation time in hours gives the daily regeneration rate of both red cells and haemoglobin in per cent. provided that in the period under consideration there is no change in colour index. Watson (1938) has suggested that it is necessary in order to estimate the regeneration rate of haemoglobin to multiply the regeneration rate of red cells by the colour index; such a calculation is wrong. Clearly if there is no change in colour index the rates of regeneration of red cells and haemoglobin must be identical. Consideration must be paid to the colour index only if the newly-produced cells contain a quite different amount of haemoglobin from that in the older cells. In such a case an accurate estimation of haemoglobin regeneration is of course impossible. Over short periods, however, the error is probably negligible if the red cell regeneration rate be multiplied by the quotient: final colour index/original colour index. Changes in the colour index do occur from time to time in the recovery stage of haemolytic anaemia and when they are encountered they demand consideration in studies of haemoglobin metabolism.

In all normal subjects and in pathological conditions with stable R.B.C. counts the daily destruction rate must be equivalent to the regeneration rate and the average life span of erythrocytes can be calculated on the same basis. The life span in days is obtained if 100 is divided by the daily regeneration (or destruction) rate. If the normal average reticulocyte count is 0·7 per cent. and, according to the investigations reported above, the average maturation time for normal reticulocytes is seven hours, the normal daily regeneration rate is $\frac{0{\cdot}7 \times 24}{7}$ or 2·4 per cent., the average life span of R.B.C. therefore is $\frac{100}{2{\cdot}4} = 42$ days. The average life span here means not only the average for normal individuals but also the average time for the red cell population of any individual; it is independent of whether the erythrocytes die of old age or are destroyed indiscriminately in certain numbers each day, or of whether the life span is identical for all cells or is variable depending on the individual resistance to wear and tear in the circulation and the continual metabolic changes.

There is, however, one hypothetical assumption in this calculation. It is assumed that none of the reticulocytes are destroyed before they mature into adult R.B.C. After all that has been said above the suggestion of Mermod and Dock (1935) that all reticulocytes are quickly destroyed in the circulation can be disregarded. In normal persons, even in the case of indiscriminate destruction, the number of reticulocytes destroyed within the short period of their maturation time would be negligible. In pathological conditions this question can only be answered if it is known whether the erythrocytes die by ageing or are destroyed indiscriminately. Experiments on blood regeneration and some transfusion experiments reported below throw some light on

this question. It may be noted here that these experiments suggest that in most conditions the majority of red cells produced at one time die after approximately the same time interval. In some cases of erythronoclastic anaemia of the newborn, however, evidence was found of indiscriminate destruction. In such conditions the real regeneration rate is higher than that calculated from our formula and the life span will be therefore shorter. Recently Rhoads and co-workers (1938) have described a haemolytic anaemia produced by the action of indol in animals kept on deficient diets. The reticulocyte counts were low in most cases. The authors assume that in this condition the reticulocytes were destroyed before they had time to mature, a suggestion which has been made also for pernicious anaemia. In the latter disease indeed only an impossibly short maturation time could account for the rate of regeneration suggested by the very large excretion of coproporphyrin I. It must therefore be admitted that in some conditions there may be an appreciable destruction of reticulocytes, but we are fairly certain that in most conditions the destruction of reticulocytes is not of significance. This belief is partly founded on the following observations: The investigations of Rous and Robertson (1917a and b) and of Doan and Sabin (1926) admit of little doubt that fragmentation of R.B.C. is a major factor in blood destruction. Fragmentocytes or schizocytes are fairly frequently seen in various erythronoclastic forms of anaemia, especially in Cooley's anaemia, in the peculiar type recently described by Parsons (1935, 1938a and b), in the type of Greppi-Micheli and in syphilitic anaemia of young babies. Yet only occasionally have we seen reticulum in such fragmented cells.

Summary

1. The properties of immature erythrocytes are discussed. The presence of substantia reticulofilamentosa is the only constant and reliable sign of immaturity of the red cell.

2. Erythrocytes are liberated from the bone marrow only in the reticulocyte stage.

3. Reticulocytes 'cultured' in various media disappear at first at a constant and then with a decreasing velocity.

4. The relation between the shape of the maturation curve and the kinetics of the reticulocytes' maturation is discussed. The decreasing velocity of the disappearance is due to an initiation of necrobiotic changes in the erythrocyte and does not represent a real decrease of the velocity of maturation. The maturation time is therefore obtained by extrapolation of the initial slope to the ordinate zero.

5. The average maturation time of normal reticulocytes is about seven hours. The maturation time in cases with reticulocytosis is usually longer, but there is no fixed relation between either the number of reticulocytes or the amount of reticular substance and the maturation time. The only condition in which a maturation time shorter than the normal was found is untreated pernicious anaemia.

6. The daily regeneration rate is obtained by multiplying the number of reticulocytes per cent. by twenty-four and dividing by the maturation time in hours. One hundred divided by this figure gives the life span of erythrocytes in days if the number of red blood corpuscles is constant. From an average number of reticulocytes 0·7 per cent. and a maturation time of seven hours an average life span of forty-two days has been deduced.

7. The regeneration rate of haemoglobin is identical with that of erythrocytes as long as the colour index remains unchanged. When such a change occurs a correction must be introduced into the formula.

THE NUTRIENT ENEMA

BY

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Since Voit and Bauer (1869) published their observations on nutrient enemata, numerous papers have been written dealing with the question of absorption from ' the rectum.' As yet, no unanimity of opinion exists regarding their value. In recent years, few contributions to the subject have been made.

The present paper is a report of investigations performed with three of the more important substances which are commonly used in nutrient enemata, namely, glucose, normal saline and predigested protein.

The absorption of glucose

The largest number of investigations on absorption from nutrient enemata have been devoted to the problem of absorption of rectal glucose, but a perusal of the literature has revealed a welter of results and conclusions.

There seems to be complete conflict of opinion regarding the changes in the blood-sugar content in fasting subjects, after the injection of a glucose enema. In a series of seven cases, Tallerman (1919) found a rise in the peripheral blood-sugar level in four cases and a fall in three cases. Rubino and Varela (1922) obtained a rise in only three out of seventeen cases. Although Levi (1927) found a rise in eleven out of his sixteen cases, he did not consider this method of administering glucose of much value. In 1932, Julesz and Winkler found a rise in twelve cases in a series of fourteen; in the same year, Scott and Zweighaft (1932) were unable to demonstrate a rise in the blood-sugar level in any one of their fifty cases. Collens and Boas (1933) found a significant rise in the blood-sugar level in twenty-four normal subjects after rectal glucose.

Many workers have found that rectal washouts, done at varying periods after the glucose enema was given, failed to yield more than a fraction of the glucose injected. Carpenter (1925) regarded this disappearance of glucose as signifying absorption. Pressman (1930), on the other hand, believed that glucose not recovered was lost through fermentation; in one experiment he showed that when 5 per cent. glucose was incubated with a suspension of faeces for seven hours only 0·5 per cent. could be recovered. In animal experiments, McNealy and Willems (1929, 1931) and Davidson and Garry (1939) found that there was no loss of glucose from loops of colon in one hour and one-and-a-half hours respectively. Ebeling (1933) confirmed these results but found that when dogs were rendered hypoglycaemic, 21 per cent. of the glucose was absorbed in two hours.

Indirect methods have also been employed. Thus an increase in the respiratory quotient following rectal glucose, indicating utilization of carbohydrate in the tissues, has been reported by Reach (1902), Bergmark (1915), Carpenter (1925), and Curry and Bargen (1935) in the human subject and by Fleming (1919) and Hari and von Halasz (1918) in animal experiments. The effect of glucose enemata on ketosis has been investigated by a number of workers with varied results. Satta (1905) and Hubbard and Wilson (1922) thought that a reduction in the excretion of ketones was affected, but Langdon Brown (1911), Mutch and Ryffel (1913) and Corkill (1936) were unable to confirm this. In the course of their experiments on the effect of rectal glucose on ketosis, Bergmark (1915) and Mutch and Ryffel (1913) found a marked fall in the excretion of nitrogen after the glucose enema: the latter workers attributed this fall to the protein-sparing action of glucose absorbed from the enema and showed that the fall was approximately equivalent to that produced by a similar amount of glucose taken by mouth. Langdon Brown (1911) was unable to demonstrate this effect.

Little help in the elucidation of this problem has been offered from the clinical aspect. Only two cases have been reported, one by Collens and Boas (1933) and one by Bauer and Monguio (1932), when glucose has been given per rectum in hypoglycaemic coma. The coma was relieved in both of these cases.

The present investigation. In this investigation rectal absorption was studied in convalescent children aged between five and thirteen years. The problem was approached in three ways. In one group of investigations the peripheral blood-sugar levels were observed before and after the rectal injection of glucose solutions in the fasting subject; secondly, in some of these cases the quantity of glucose recovered from a rectal washout at the end of the test period was determined. In the third group of investigations, the effect of rectal glucose on the nitrogen metabolism was studied.

BLOOD-SUGAR STUDIES. The fasting subject was given a cleansing enema one hour before the start of the experiment when a specimen of capillary blood was obtained from the ear or the finger. The glucose solution was then allowed to run into the lower bowel through a No. 8 (English gauge) rubber

catheter inserted into the rectum. In subsequent investigations all fluids administered rectally were given in this way. The enemata were all retained and did not give rise to discomfort except when 20 per cent. glucose solutions were given. With these the subjects had difficulty in retaining the fluid and in a few cases colic was experienced. Further specimens of blood were taken 30, 60, 90 and 120 minutes after the commencement of the experiment. In the great majority of the cases there was a fall in the blood-sugar percentage after the enema and in only one was there a significant rise; this occurred in one of the children who received 20 per cent. glucose. In table 1 the maximum and minimum values found and the mean figure, representing the composite curve for each group, are shown.

If glucose is absorbed from the rectum in any quantity it seems likely that there will be a rise in the blood-sugar level as occurs when it is taken by the mouth. On the other hand it is possible that the glucose is absorbed so slowly that the liver is able to store it and thus prevent its entrance into the general circulation. Subsequent experiments tend to show that this is the probable explanation of the failure of the glucose enema to cause a rise in the blood-sugar level.

THE GLUCOSE CONTENT OF THE RECTAL WASHOUT. In fifteen cases the rectum was washed out at the end of the experiment and the returned fluid examined for glucose. There was great variation in the amount of glucose recovered. The percentage of the instilled glucose recovered varied from 7·45 per cent. to 60 per cent. It is impossible to say whether the loss of glucose represented by these figures was due to absorption from the bowel, to incomplete recovery of unabsorbed material, or to fermentation. There is, however, some evidence to show that neither fermentation nor loss of glucose due to incomplete recovery plays an important part.

In their work on patients with colostomies Curry and Bargen (1935) found a similar disappearance of glucose after the solution had been allowed to remain in the lower colon for three hours. Further evidence that the recovery of unabsorbed material by a rectal washout is a reasonably accurate procedure is found in the work of Voit and Bauer (1869). In the course of their studies on the absorption of nutrient enemata, muscle albumin was given per rectum; some hours later, a cleansing enema failed to recover 29 gm. of the substance, but an equivalent amount of nitrogen appeared in the urine as urea. It is probable that some degree of fermentation does take place, since several of the normal flora of the colon ferment glucose.

I have found that when glucose solutions were incubated with suspensions of faeces at 37° C. for two hours, the loss of glucose was never more than 10 per cent.; in one experiment, it was found that the loss in twenty-four hours was only 20 per cent. Fermentation of glucose and incomplete recovery of unabsorbed material due to inefficiency of the washout cannot account for all the glucose lost. It follows, therefore, that some significance must be attached to the results obtained by this method.

THE EFFECT OF THE GLUCOSE ENEMA ON NITROGEN METABOLISM. In view of the inconclusive results obtained from blood-sugar studies and from estimation of the residual glucose in the rectal washout the problem of glucose absorption from the rectum was approached indirectly by determining what effect the enema had on the nitrogen metabolism. It is well known that if a subject on a low carbohydrate diet is given additional carbohydrate there is reduction in the amount of nitrogen excreted, the carbohydrate having a sparing effect on nitrogen metabolism. If there is absorption from the rectum, glucose given by that route should have this effect in the same way as it has when given by the mouth. This was tried in six subjects. They were given a

TABLE 1

BLOOD-SUGAR CONTENT AFTER GLUCOSE PER RECTUM

No. of cases	Glucose enema			Blood-sugar in mgm. per 100 c.c.				
	Vol. c.c.	Glucose per cent.		Fasting	30 min.	60 min.	90 min.	120 min.
10	200	5	Maximum	112·3	97·1	96·1	91·7	90·9
			Minimum	47·6	41·0	37·3	38·9	34·7
			Mean	75·9	68·4	65·6	68·6	67·4
10	200	10	Maximum	111·1	116·0	79·3	83·3	78·7
			Minimum	56·5	50·0	55·5	52·6	52·6
			Mean	72·9	70·2	65·9	64·5	65·8
10	200	13·5	Maximum	96·2	95·3	90·9	86·9	87·9
			Minimum	59·3	60·3	62·5	64·1	60·9
			Mean	82·5	81·5	78·4	75·8	78·7
5	200	20	Maximum	100·0	80·0	117·6	125·0	125·0
			Minimum	78·7	66·6	66·6	62·5	63·3
			Mean	86·9	74·6	83·7	81·6	81·7

diet of a caloric value sufficient for basal and energy requirements but in which the carbohydrate portion was limited to a quantity low enough to make it mildly ketogenic. The daily output of nitrogen was estimated from twenty-four-hour specimens of urine which had been preserved under toluol after collection. All the cases showed definite diminution in nitrogen excretion after the glucose enema, but unfortunately in four of the subjects it was impossible to attain a sufficient constancy in the nitrogen excretion to determine whether the reduction observed was accidental or caused by the sparing action of glucose. In two, however, fairly constant excretion was attained, and after the enema there was a quite definite fall for two or three days with a subsequent rise to the level found in the pre-period (table 2).

Conclusions. Glucose is absorbed from a simple solution in water, introduced into the lower bowel. The amounts of glucose absorbed vary considerably.

Concentrations of 5, 10, and 13·5 per cent. were well borne. Definite signs of irritation followed the use of 20 per cent. solutions. It is not possible to say from this investigation, what is the optimum concentration.

The rectal absorption of sodium chloride

One of the commonest procedures in post-operative surgical treatment is the administration of rectal saline, either by small repeated enemata or by continuous proctolysis. In most modern text-books of physiology, the statement is made that salts are absorbed by the large bowel, yet there appears to be little experimental evidence to support this claim. A search of the literature on the subject of absorption from nutrient enemata has revealed only four works dealing with the absorption of rectal saline.

In 1869, Voit and Bauer found that sodium chloride was excreted quantitatively in the urine of a dog after the animal had been given a solution of sodium chloride by rectum. Nakazawa (1925) and McNealy and Willems (1929) also found evidence of absorption of sodium chloride from the colon in experimental animals. Gompertz (1910) confirmed these observations in the human subject.

The present investigation. Two children were put on a constant diet to which no salt had been added in the cooking. By this means a fairly constant salt intake was ensured. In order to provide an abundant maintenance supply of chloride, however, 2 gm. of sodium chloride were added to the diet each day. The daily output of urine was collected under toluol and its chloride content estimated. After a control period case 1 received an additional 5 gm. of sodium chloride by mouth daily for three days; two days were allowed after this without extra salt for the excretion of chloride to regain its original level, and then 600 c.c. normal saline (0·9 per cent. NaCl) were given by rectum daily for a further period of three days. During this period the child was given a cleansing enema early each morning; 300 c.c. of the saline were instilled at 10 a.m. and 6 p.m. Case 2 did not have any extra chloride by mouth, but after a control period received 600 c.c. normal saline per rectum

TABLE 2

THE INFLUENCE OF RECTAL GLUCOSE ON NITROGEN METABOLISM

Case 5 (J. M.) aet. 7 years

DIET
Protein .. .. 55 gm.
Carbohydrate .. .. .. 45 gm.
Fat .. .. 85 gm.
Calories 1165
AK/K Ratio .. .. .. 1/1·6
Glucose enema: 15 gm. glucose in 300 c.c. water

Date	Volume of urine c.c.	Total urinary nitrogen gm.
17/18	630	10·39
18/19	510	10·29
19/20	620	11·54
20/21	540	10·32
21/22	740	10·91
22/23	910	9·70
23/24	770	9·98
24/25	500	9·77
25/26	520	10·75
26/27	570	11·79
27/28	550	10·67

Enema 10 a.m. 21st
6 p.m. 21st
10 a.m. 22nd
6 p.m. 22nd

Case 6 (J. C.) aet. 10 years

DIET
Protein .. .. 47 gm.
Carbohydrate .. .. .. 51 gm.
Fat .. .. 109 gm.
Calories 1237
AK/K Ratio .. .. .. 1/1·8
Glucose enema: 15 gm. glucose in 300 c.c. water

Date	Volume of urine c.c.	Total urinary nitrogen gm.
25/26	660	12·40
26/27	690	12·43
27/28	690	12·75
28/1	725	13·03 *
1/2	725	13·03 *
2/3	1230	11·14
3/4	740	11/68
4/5	740	13·96
5/6	960	13·47
6/7	950	12·56

Enema 10 a.m. 1st
6 p.m. 1st
10 a.m. 2nd
6 p.m. 2nd

* Specimens of the 28/1 and 1/2 were pooled since 150 c.c. of the former specimen had been added in error to the following day's collection.

on each of three days. The enemata were all well tolerated and none was rejected.

Details of the results are given in tables 3 and 4. In case 1 a nicely balanced excretion of chloride was obtained, and during periods 2 and 3 where extra chloride was given in the former period by mouth and in the latter by rectum, approximately the same quantity of chloride was excreted in the urine in each period. In period 2 the daily average of chloride excreted was 3·43 gm. more than the daily average of the control period and in period 3 it was 3·71 gm. in excess. Diuresis was not marked in this case. In case 2 it was found that there was considerable variation in the quantity of chloride excreted from day to day. Because of this, the prolonged control period of eleven days was deemed necessary. During the period in which rectal saline was given, the amount of chloride excreted daily rose above that excreted on the peak days of the control period and the average daily output was 13·16 gm. whereas the average for the control period was 5·58 gm. per day. Thus, more chloride was excreted in period 2 than was given in the food and in the rectal infusion. A marked diuresis took place during this period (approximately 600 c.c. per day), and it is probable that the increase in the chloride excretion was in some part due to this.

TABLE 3

RECTAL ABSORPTION OF SODIUM CHLORIDE

CASE 1 (C. R.) Aet. 9 years

DIET

Protein	60 gm.
Carbohydrate ..	146 gm.
Fat	63 gm.

	Date	Volume of urine c.c.	Sodium chloride gm.
PERIOD 1	13/14	700	5·72
Control	14/15	1150	6·17
	15/16	520	5·65
PERIOD 2	16/17	1040	9·97
5 gm. NaCl by mouth	17/18	820	9·09
mouth daily	18/19	960	8·18
	19/20	710	4·34
	20/21	400	2·58
PERIOD 3	21/22	1160	8·36
600 c.c. normal saline	22/23	900	10·07
per rectum daily	23/24	920	9·76

TABLE 4

RECTAL ABSORPTION OF SODIUM CHLORIDE

CASE 2 (J. L.). Aet. 8 years

DIET

Protein	60 gm.
Carbohydrate	146 gm.
Fat	63 gm.

Date	Volume of urine c.c.	Sodium chloride gm.	
4/5	1025	8·85	
5/6	520	4·81	
6/7	750	5·89	
7/8	510	3·28	
8/9	540	3·20	
9/10	650	4·54	
10/11	910	8·28	
11/12	850	7·91	
12/13	510	4·24	
13/14	540	5·49	
14/15	690	4·87	
15/16	1130	12·43	600 c.c. normal saline
16/17	1630	14·72	daily during this period
17/18	1190	12·40	
18/19	940	8·75	
19/20	1000	7·20	
20/21	860	7·23	

Conclusions. Normal saline, when given rectally, is easily retained and is non-irritant. Chloride is readily absorbed from a rectal infusion of normal saline.

Absorption of predigested protein

The nutrient enema of former times was rather elaborate. It frequently contained eggs, bouillon and honey, sometimes wine.

As long ago as 1668 Regnier de Graaf (Friedenwald and Morrison, 1940), from clinical observations, expressed doubts as to the efficacy of such mixtures, but their use has continued into modern times. The protein content of the nutrient enema was considered of paramount importance, and when modern research took up the problem of absorption from nutrient enemata, the question of protein absorption was the first to be attacked. Voit and Bauer (1869) considered that egg albumen, muscle and peptone were well absorbed, but were of opinion that the procedure had no practical value. Ewald (1887) was convinced that a considerable amount of nourishment could be given in this way. He believed that predigested food was not necessary. Boyd and Robertson (1906) thought that little protein was absorbed from an enema although they believed that considerable quantities of sugar and fats were absorbed. Langdon Brown (1911) found no increase in the nitrogen content of the urine after an enema of milk, dextrose, sodium bicarbonate and liquor pancreaticus; however, this mixture was incubated for only twenty minutes before its administration. Mutch and Ryffel (1913) found that a rectal washout yielded only 0·34 gm. nitrogen after an enema of peptonized milk containing 2·8 gm. nitrogen but finding indican in the patient's urine, they concluded that considerable fermentation had taken place. In a series of balance experiments, Short and Bywaters (1913) gave rectal injections of peptonized and of pancreatized milk. No increase in the nitrogen excretion followed the taking of the peptonized milk, a small increase followed the injection of the milk pancreatized for twenty minutes and the maximum increase was found after milk treated for twenty-four hours was given.

In the course of an investigation on liver function Gottschalk and Nonnenbruck (1923) compared the effects of giving a mixture of amino-acids by mouth

and by rectum. They found that whereas after oral ingestion, there was a rise in the non-protein nitrogen of the blood but no increase in the amino-acid nitrogen of the urine, there was a definite rise in the amino-acid nitrogen of the urine after the mixture was given rectally. Nakazawa (1925) reported evidence of absorption of glycine from the large bowel in anaesthetized animals.

It appears that although the older workers believed that native protein was well absorbed from the enema, later workers considered that absorption only took place from an enema of protein that had been predigested or from one consisting of amino-acids.

The present investigation. Three children were put on high protein diets. These diets contained over 2 gm. of protein per kgm. of body weight. Urine was collected in twenty-four-hour specimens, preserved under toluol and analysed for total nitrogen. When nitrogen excretion had become constant, three periods each of three days were studied, in the second of which the patient received predigested protein per rectum. During each day of the second period a cleansing enema was given in the early morning, followed by a nutrient enema at 10 a.m. and again at 6 p.m.

The nutrient enema was prepared in the following manner. A quantity of casein soluble (B.P.C.) was mixed with a hundredth of its weight of trypsin powder and triturated with distilled water. This mixture was placed in the incubator overnight (about eighteen hours) and the next morning boiled before use. The pH of this mixture, after incubation, was found to be between 7 and 7·5. In order to determine the amount of protein that had been converted into amino-acid a sample was treated with 15 per cent. trichloracetic acid to precipitate any undigested protein and then the total nitrogen of the filtrate determined. The enemata were all retained and did not produce any irritation.

Results. Details of each study are given in table 5. From this, it can be seen that in each case the excretion of nitrogen rose during period 2 by an amount approximately equal to the amount of non-protein nitrogen given per rectum. It would appear, therefore, that the non-protein nitrogen was absorbed. Under the conditions of this study considerable quantities of nitrogenous material were absorbed from enemata.

An objection to giving protein per rectum was raised by Voit and Bauer (1869). They argued that the amounts absorbed were so small that as a practical procedure such an enema was useless, since the absorption of a small amount of nitrogenous material stimulated the catabolism of more protein than it replaced. In this study, the amounts of nitrogen absorbed were equivalent to 12–13 gm. of protein per day. The amounts given were chosen merely for convenience, but after observing how well the predigested casein enemata were tolerated, it seems likely that it is feasible to give sufficient quantities to be a practical value in nutrition.

Conclusions. Non-protein nitrogen is absorbed quantitatively from an enema of predigested casein. It is apparently metabolized in a similar manner to nitrogenous food given by mouth.

TABLE 5

RECTAL ABSORPTION OF PREDIGESTED CASEIN

		Case 1 (J. R.)			Case 2 (T. P.)			Case 3 (S. R.)		
DIET		Protein .. .. 65 gm. Carbohydrate .. 92 gm. Fat .. .. 94 gm.			Protein .. .. 103 gm. Carbohydrate .. 127 gm. Fat .. .. 104 gm.			Protein .. .. 88 gm. Carbohydrate .. 172 gm. Fat .. .. 93 gm.		
ENEMA		Casein soluble .. 8·40 gm. Trypsin .. 0·084 gm. Water .. .. 250 c.c.			Casein soluble .. 10 gm. Trypsin .. 0·1 gm. Water .. .. 300 c.c.			Casein soluble .. 10 gm. Trypsin .. 0·1 gm. Water .. .. 300 c.c.		
Total non-protein nitrogen of enemata ..		5·94 gm.			6·6 gm.			6·43 gm.		
Period	Day	Vol. of urine c.c.	Total N gm.	Total N for each period gm.	Vol. of urine c.c.	Total N gm.	Total N for each period gm.	Vol. of urine c.c.	Total N gm.	Total N for each period gm.
1	1	510	7·30		810	11·76		630	9·63	
	2	580	7·63	23·20	710	12·20	38·02	710	11·60	30·55
	3	620	8·27		750	14·06		620	9·32	
2	4	660	11·19		880	15·40		870	10·80	
	5	840	8·64	28·59	1300	17·60	44·20	1120	13·76	35·23
	6	780	8·76		730	11·20		830	10·67	
3	7	650	8·61		930	14·78		700	10·42	
	8	600	7·03	26·22	500	8·95	—	710	11·89	30·74
	9	780	10·58		560 *	6·16		500	8·43	

* Urine lost from this specimen

Biochemical methods

Blood-sugars were estimated by the method of Folin and Wu as modified by Herbert and Bourne (1931), and the residual glucose in the rectal washout by Benedict's (1911) method for sugar in the urine.

The chloride content of the urine was estimated by Van Slyke's (1924) method.

Total nitrogen was estimated by the macro-Kjeldahl method.

Summary

1. Glucose, sodium chloride and predigested protein are absorbed from solutions introduced into the lower bowel.

2. There is great variation in the amount of glucose absorbed.

3. Absorption of sodium chloride, when given by the rectum, is almost as great as it is when given by mouth.

4. Considerable quantities of predigested protein are absorbed from the enema.

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ABSORPTION OF DRUGS FROM THE RECTUM

BY

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The administration of drugs by the rectum has been practised from the earliest times. Prescriptions for suppositories were found in the Ebers Papyrus (Bryan, 1930). It would appear that these suppositories were intended for the local treatment of disease of the proctodeum. In more recent years, the idea arose that the rectal route could be used for systematic medication, either as supplementary to the oral route or as an alternative when medicine could not be given by mouth. Cullen appears to have been among the first to realize the wider possibilities of rectal medication (Thomson, 1827). In describing the treatment of tetanus, he wrote that large quantities of opium should be employed before deglutition becomes difficult, 'or that, if this opportunity be lost, the medicine, in sufficient quantity, and with due frequency, should be thrown into the body by clyster; which does not seem to have been hitherto often practised.'

In modern times, although hypodermic, intramuscular and intravenous injections afford a sure means of supplementing or supplanting the oral administration of medicine, the rectal route is still of value. Of the drugs given rectally, clinical evidence of absorption is conclusive in a number of instances, but many drugs which are given per rectum produce no easily recognized effects and clinical evidence of their absorption is equivocal. There are few controlled studies on the rate and completeness of absorption of rectally administered drugs. In the present study absorption of bromide, salicylate and sulphanilamide after rectal administration has been investigated.

Rectal absorption of potassium bromide

It has been stated frequently that bromide is absorbed when administered rectally, but it has proved impossible to find any records of experimental work substantiating this opinion. It is probable that simple bromide salts are well absorbed, for the more complex substance, tribromethylalcohol (avertin), is definitely absorbed and now holds an established place in the realm of anaesthetics. Previous researches have shown that in the human subject two other halogen salts are well absorbed by the rectum. In an earlier paper (Mackenzie, 1943), Gompertz's (Carpenter, 1925) observation that chloride was well absorbed was confirmed, and Gompertz, Osborne (1922) and Cohn (1932) have shown that iodide is absorbed readily when given in solution.

The present investigation. A one-per-cent. solution of potassium bromide in water was given per rectum to five children. In each the enema was completely retained. The urine was collected for several days and analysed for bromide. The results are shown in table 1.

There was some variation from case to case in the quantities recovered, though the amount excreted in the urine never represented more than a small proportion of the total bromide given. From 7·1 to 11·3 per cent. was recovered in the forty-eight hours after the bromide was given. In 1908, Hales and Fishman gave single doses of sodium bromide by mouth to three students. The bromide excreted in the urine was estimated for several days thereafter. The results obtained by these workers are strikingly similar to these of the present investigation where the bromide was given per rectum. In one case when 1 gm. of sodium bromide was given, Hales and Fishman (1908) found that 8·29 per cent. appeared in the urine in forty-eight hours; in the other two, when 2 gm. of sodium bromide were given, 10·8 per cent. was recovered from one in forty-eight hours and in the second 11·3 per cent. was recovered in fifty-six hours.

The slow excretion of bromide was shown by

TABLE I

THE EXCRETION OF BROMIDE IN THE URINE AFTER RECTAL ADMINISTRATION

Case	Age in years	KBr given (gm.)	Bromide excreted (as mgm. KBr)				
			Before enema	1st day	2nd day	Total for 48 hours	Per cent. recovered
E. H.	9	1	nil	43·0	28·3	71·3	7·1
S. D.	5	2	nil	80·2	116·2	196·4	9·8
J. R.	8	2	nil	58·3	104·0	162·3	8·1
M. C.	6	2	nil	133·3	45·8	179·1	8·9
E. L.	4	2	nil	95·2	132·0	227·2	11·3

Bill (1869) to be due to the fact that after absorption it displaces some of the chloride of the body fluids. The bromide is only excreted when more chloride is absorbed. So it may be assumed that there is no material difference in the amounts excreted in the urine when the salt is given in solution per rectum from those when a similar dose is given by mouth.

Conclusion. Potassium bromide is absorbed as readily from the rectum as it is when given by the mouth.

Rectal absorption of sodium salicylate

The use of salicylates for the control of pain and temperature in acute rheumatic arthritis is well recognized. With the large doses now commonly employed, toxic manifestations and gastric disturbances have been observed frequently.

In an attempt to minimize these undesirable effects, Heyn (1912) gave sodium salicylate per rectum. He showed that the instillation of 120 to 240 grains of sodium salicylate in 6 oz. of starch water was followed by the relief of the joint pain and a fall in the temperature. Since these therapeutic effects were achieved by rectal salicylate, Heyn (1912) considered the rectal route preferable to the oral one, as thereby gastric disturbances were avoided. Irving (1923) reported similar experiences in children; employing a dosage of 1 to 3 grains per pound of body weight, he noted that older children occasionally complained of headache and tinnitus within fifteen to forty-five minutes of the instillation of the sodium salicylate. Recently Valesquez (1939) obtained satisfactory results with an isotonic solution of sodium salicylate (23·2 gm. per litre.) He has reaffirmed that, since gastric disturbances are thereby avoided, the rectal route is preferable to the oral one.

No controlled study has been reported on the rectal absorption of salicylate in the human subject. In 1933, Blume and Nohara compared the level of salicylate in the blood and the quantity excreted in the urine of rabbits after oral and rectal administration. A slightly higher blood salicylate content was found after rectal administration than when the drug was given by mouth. Fifty to 80 per cent. of salicylate was recovered in the urine whatever the method of administration. They believed that absorption was more rapid when the drug was given per rectum than by the mouth, as the solution came immediately into contact with a large area of mucosa from which absorption could take place.

The present investigation. One gramme of sodium salicylate and 2·5 gm. of sodium bicarbonate dissolved in 200 c.c. of water were given rectally to ten children. Ten others were given the solution in the same quantities by mouth. The total excretion of salicylate in the urine voided in the thirty-six hours following the administration of the salicylate solution was estimated in each case. Salicylate given by mouth seems to be excreted for a variable time; Hanzlik et al. (1917) gave seventy-eight hours as a median for normal subjects. In the present series most of the salicylate was excreted in twenty-four hours: with two exceptions, only a trace of salicylate was found in the urine excreted from twenty-four to thirty-six hours after administration by mouth or by rectum. Results are given in tables 2 and 3.

The quantities of salicylate recovered in the urine varied considerably in both series. Where the solution was given by mouth, between 10·8 per cent. and 64·9 per cent. were recovered. On the other hand, when it was given per rectum, between 17 per cent. and 52·5 per cent. was recovered. The average recovery was approximately the same in each series, after oral administration 34·4 per cent. and after rectal administration 33·4 per cent.

Most workers agree that salicylate is incompletely excreted, a part being destroyed in the tissues. The amount excreted appears to vary under different conditions, and even under the same conditions there is some variation in the figures given by different workers. When it was given over a period of several days, Hanzlik and Wetzel (1919) found that the total salicylate excreted in the urine varied between 75 per cent. and 80 per cent. On the other hand, Morris and Graham (1931) found that over a period of six days, only 26·0 to 37·6 per cent. of the ingested salicylate was recovered in the urine, but when sodium bicarbonate was given along with the salicylate, the urinary excretion rose to 80 to 91·4 per cent. Apparently the fraction of salicylate

TABLE 2

RECOVERY OF SALICYLATE FROM THE URINE AFTER ORAL ADMINISTRATION OF 1 GM. SODIUM SALICYLATE

Case	Age in years	0–4 hr.		4–24 hr.		24–36 hr.		Total salicylate recovered per cent.
		Vol. c.c.	Sod. Sal. gm.	Vol. c.c.	Sod. Sal. gm.	Vol. c.c.	Sod. Sal. gm.	
D. McQ.	9	350	0·286	700	0·363	210	nil	64·9
M. McE.	8	250	0·106	400	0·156	300	nil	26·2
G. W.	5	400	0·034	300	0·208	900	nil	24·2
J. R.	10	480	0·106	860	0·327	960	nil	45·3
M. C.	6	300	0·131	500	0·040	550	nil	17·1
R. C.	6	300	0·200	100	0·006	500	nil	20·6
S. L.	12	225	trace	260	0·200	80	nil	20·0
H. P.	7	500	0·278	500	0·417	—	nil	69·5
J. R.	10	70	0·064	240	0·044	500	trace	10·8
H. McD.	9	210	0·200	2200	0·275	—	nil	47·5
Average		308·5	0·140	580	0·204			34·4

TABLE 3

RECOVERY OF SALICYLATE FROM THE URINE AFTER RECTAL ADMINISTRATION OF 1 GM. SODIUM SALICYLATE

Case	Age in years	0–4 hr.		4–24 hr.		24–36 hr.		Total salicylate recovered per cent.
		Vol. c.c.	Sod. Sal. gm.	Vol. c.c.	Sod. Sal. gm.	Vol. c.c.	Sod. Sal. gm.	
G. McL.	8	350	0·213	320	0·089	700	nil	30
,,	8	410	0·096	990	0·421	520	nil	52
J. C.	7	65	0·028	320	0·267	800	0·080	38
A. C.	6	70	0·015	400	0·240	120	nil	25·5
P. S.	5	80	0·027	200	0·159	110	trace	18·4
P. B.	9	325	0·030	700	0·495	250	trace	52·5
M. H.	8	85	0·014	250	0·131	180	0·100	24·5
J. B.	10	410	0·156	480	0·385	320	nil	54·1
H. S.	10	520	0·297	525	0·091	—	nil	39
A. M.	11	150	0·057	535	0·110	—	nil	17
J. B.	5	—	—	—	0·274	—	trace	27·4
Average		247	0·094	475	0·240			33·4

excreted in the urine is much smaller when single small doses are given. Devrient (1921) recovered only from 1·01 to 14·68 per cent. in the urine of patients who each had been given 1 gm. of sodium salicylate in a single dose.

A comparison of the results of the present investigation shows that there is no significant difference in the quantities of salicylate recovered in the urine after oral or rectal administration of sodium salicylate. Thus, it may be assumed that there is no difference in the degree of absorption when the drug is given in solution by either route. A comparison of the rate of excretion of salicylate given by the mouth with the rate when given by the rectum shows that excretion is more rapid after oral administration. The average amount excreted in four hours when the oral route was employed was 0·14 gm., while after rectal injection 0·094 gm. was excreted in the same time. This fact suggests that absorption may be slower when the drug is given per rectum. It is possible that diuresis may also be a factor in determining the rate of excretion. In comparing the two series, it was noticed that where a greater quantity of salicylate was excreted in the first four hours after the salicylate solution was given there was also a greater volume of urine excreted, but that diuresis was greater after the drug was given by the mouth than it was after it was given by the rectum.

Conclusions. Sodium salicylate is readily absorbed from a rectal infusion.

The quantities of salicylate which are excreted after single doses of sodium salicylate given either by mouth or by rectal infusion are variable. Much of the absorbed salicylate is apparently destroyed in the tissues.

Excretion is rapid after both oral and rectal administration, but it is more rapid when given by the mouth than by the rectum.

As a therapeutic measure salicylate enemata can, if necessary, replace the oral administration of the drug.

Rectal absorption of sulphanilamide

In the administration of sulphanilamide by the mouth nausea and vomiting are frequent accompaniments and the employment of alternative methods may be necessary. Under such conditions, rectal administration as a practical alternative to the painful and sometimes irritating intramuscular injection suggests itself.

That rectal administration of sulphanilamide may be a procedure of practical importance is suggested by the work of Turell, Marino and Nerb (1940). These workers obtained a concentration of 9–15 mgm. per cent. total sulphanilamide in the blood after the rectal instillation of a 1 per cent. solution of the drug over periods of from twenty-four to sixty-five hours. When suppositories were used, the maximum concentration obtained in the blood was 6 mgm. per cent. Wood (1941) reported similar observations with a saturated solution of sulphanilamide. In one case, he obtained a blood sulphanilamide concentration of 12·5 mgm. per cent. after giving 250 grains of sulphanilamide per rectum.

The present investigation. The absorption of the drug when given by the mouth and by the rectum has been compared. The concentration of free sulphanilamide in the blood and the excretion of total sulphanilamide in the urine have been estimated after giving the drug by the mouth in solution and by the rectum when given in solution and in the form of suppositories.

One gramme of sulphanilamide dissolved in 200 c.c. of water was given by mouth to each of ten children. The free sulphanilamide content of specimens of capillary blood taken at one, two, three and five hours after the drug had been given was determined. The total sulphanilamide was estimated in the urine collected for thirty-six hours. A second group of ten children each received 1 gm. of sulphanilamide in 200 c.c. of water per rectum and a third group two suppositories, each containing

0·5 gm. sulphanilamide: specimens of blood and urine were collected and the sulphanilamide content estimated in each case. The results are shown in tables 4, 5 and 6.

Where the sulphanilamide solution was given by mouth, the levels of free sulphanilamide in the blood, after rising to a maximum at one hour, showed a progressive fall, though at five hours there were still considerable quantities of free sulphanilamide in the blood. The recovery in the urine was incomplete, the quantities recovered varying from 76·8 per cent. to 25 per cent. of the amount ingested (average 50·1 per cent.).

Following the rectal infusion of the sulphanilamide solution, the free sulphanilamide content of the blood rose, in some cases, to between 3 and 4 mgm. per cent. The curve was, however, of a different shape, the peak being reached at between two and three hours after the sulphanilamide was given. In all but one case, there was still considerable free sulphanilamide present in the blood at the end of five hours. The amount recovered from the urine varied from 20·9 to 52·5 per cent. with an average of 42·9 per cent.

Following the administration of the suppositories, in only two cases was there sufficient free sulphanilamide present in the blood in the ensuing five hours for an accurate quantitative estimation to be made. The average amount recovered in the urine was little more than half of that recovered when the drug was given in solution by the rectum (24·5 per cent.).

From a study of the first group, it is evident that both absorption and excretion are rapid when sulphanilamide is given in solution by mouth. The average figure for recovery of total sulphanilamide is similar to that obtained by Scudi and Ratish (1938) in three adult cases. In their investigation the drug continued to be excreted in the urine for fifty hours. In this study upon children, the drug

TABLE 4

ABSORPTION AND EXCRETION OF SULPHANILAMIDE AFTER ORAL ADMINISTRATION OF 1 GM SULPHANILAMIDE

Case	Age in years	Blood Free sulphanilamide mgm. per cent.				Urine Total sulphanilamide	
		1 hr.	2 hr.	3 hr.	5 hr.	Mgm.	Per cent. recovered
G. W.	5	7·0	5·0	5·3	4·6	768	76·8
M. L.	7	—	4·7	4·4	3·7	744	74·4
J. L.	7	5·0	5·0	4·7	3·8	694	69·4
A. R.	9	5·3	4·0	3·0	2·8	500	50·0
J. D.	11	3·2	—	—	—	556	55·6
C. D.	8	5·0	4·0	3·2	2·0	315	31·5
J. L.	8	6·1	4·4	3·8	2·9	250	25·0
E. K.	5	5·7	5·0	4·4	3·6	323	32·3
M. McC.	7	6·3	5·4	4·7	4·0	384	38·4
M. Q.	12	5·3	3·6	3·0	2·9	500	50·0
Mean values		5·4	4·5	4·1	3·4	501	50·1

TABLE 5

ABSORPTION AND EXCRETION OF SULPHANILAMIDE AFTER 1 GM. SULPHANILAMIDE IN 200 C.C. WATER PER RECTUM

Case	Age in years	Blood Free sulphanilamide mgm. per cent.				Urine Total sulphanilamide	
		1 hr.	2 hr.	3 hr.	5 hr.	Mgm.	Per cent. recovered
M. McE.	8	2·2	2·8	3·6	4·2	277	27·7
A. C.	6	2·8	3·9	3·6	2·9	500	50·0
A. B.	12	3·1	3·4	2·5	2·6	209	20·9
G. W.	5	3·2	3·1	3·6	2·2	625	62·5
M. L.	7	2·1	2·9	3·9	3·1	591	59·1
J. L.	7	1·9	2·7	3·1	3·0	236	23·6
A. R.	9	2·5	3·0	2·7	2·5	515	51·5
J. L.	8	1·5	2·0	1·9	1·7	454	45·4
E. K.	5	2·0	2·8	2·2	F. tr.	250	25·0
R. S.	8	2·5	2·7	2·9	2·3	528	52·8
Mean values		2·4	2·9	3·0	2·7	429	42·9

TABLE 6

ABSORPTION AND EXCRETION OF SULPHANILAMIDE AFTER TWO 0·5 GM. SULPHANILAMIDE SUPPOSITORIES

Case	Age in years	Blood Free sulphanilamide mgm. per cent.				Urine Total sulphanilamide	
		1 hr.	2 hr.	3 hr.	5 hr.	Mgm.	Per cent. recovered
G. W.	5	F. Tr.	1·3	1·6	2·0	526	52·6
M. McC.	7	F. tr.	F. tr.	F. tr.	F. tr.	181	18·1
C. R.	9	nil	F. tr.	Tr.	Tr.	111	11·1
J. L.	8	Tr.	Tr.	Tr.	Tr.	95	9·5
A. McD.	8	F. tr.	Tr.	Tr.	—	238	23·8
M. H.	8	F. tr.	Tr.	Tr.	Tr.	357	35·7
M. C.	10	F. tr.	Tr.	Tr.	2·1	436	43·6
E. M.	10	F. tr.	Tr.	Tr.	Tr.	156	15·6
M. S.	11	F. tr.	Tr.	Tr.	Tr.	109	10·9
M. L.	7	F. tr.	Tr.	Tr.	Tr.	157	15·7
Average						245	24·5

could no longer be recovered from the urine after thirty-six hours.

The figures obtained from estimations of sulphanilamide in the blood and in the urine after the rectal injection of a sulphanilamide solution indicate that it is readily absorbed from the enema. A comparison of the composite blood sulphanilamide curves obtained after the oral and the rectal administration of the drug shows that absorption by the rectal route is definitely slower than when it is given by mouth. The fact that smaller quantities of sulphanilamide are recovered in the urine after rectal administration suggests that absorption may not be as complete as when it is given by mouth.

The failure to find more than traces of free sulphanilamide in the blood after the administration of suppositories shows that absorption is much slower when the drug is given rectally in a solid form; for absorption to take place, solution is necessary. In the hind gut, one of the principal functions is the absorption of water, and under normal conditions the contents of the rectum are relatively dry. Unless fluid is supplied, solution and absorption must be slow. That absorption of sulphanilamide from suppositories is less efficient is shown by the small amount recovered in the urine.

In treatment with this drug it is justly held that it is important to attain a high concentration in the blood as soon as possible. The foregoing results suggest that of the methods studied, oral administration attains this object most effectively. In addition to this, estimation of urinary excretion suggests that absorption when the drug is given by the rectum is not as complete as it is when given by the mouth. In the main it appears that oral administration is the method of choice, but on occasion rectal administration of the drug may find a place. Larger doses should be given when the rectal route is used. The use of suppositories appears to be of little value.

Conclusions. Sulphanilamide, given in solution by mouth, is absorbed with great rapidity. Apparently it is excreted more rapidly in children than in adults.

Sulphanilamide is readily absorbed from the rectum when given in solution, but more slowly and less completely than when given by the mouth. The drug is poorly absorbed from suppositories.

The administration of sulphanilamide in solution per rectum may be of considerable value, especially when there is intolerance to the drug given by the mouth.

General summary

1. The absorption of potassium bromide, sodium salicylate and sulphanilamide given per rectum has been studied.

2. It has been shown that when given in solution, all these substances are readily absorbed from the rectum. Absorption from sulphanilamide suppositories is poor.

3. Rectal absorption of sulphanilamide is slower than when the drug is given orally.

Biochemical methods. The bromide content of the urine was estimated by the method of Behr, Palmer and Clarke (1930). Salicylate was determined by the method of Thoburn and Hanzlik (1915). The free and total sulphanilamide was determined by the method of Bratton and Marshall (1939).

Thanks are due to Professor Geoffrey B. Fleming for his help and criticism. Part of the expenses of this investigation were defrayed by a grant from the Medical Research Council. Some of the sulphanilamide was kindly supplied by Messrs. May and Baker, Ltd., and the suppositories by the British Drug Houses, Ltd.

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INTRATHORACIC CYSTS OF INTESTINAL AND BRONCHIAL STRUCTURE

BY

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Mediastinal cysts of intestinal and bronchial structure are rarities; one example of each is here reported, since it is only by an accumulation of records of such cases that knowledge can be gained as a basis for diagnosis and treatment, and a fatal outcome prevented. They belong to a group of mediastinal cysts of embryonic origin of which the most common and best known is the dermoid. Two characteristics mark them as distinct from the dermoids: first their posterior position in the mediastinum and secondly the early age at which they cause symptoms.

Development. At what stage of development of the embryo and by what process the various types of mediastinal cyst arise is undecided. Relative to those of entodermal origin with which this paper deals, it is known that in the period when the human embryo measures between 3 mm. and 7 mm. lateral grooves appear at one part of the primitive foregut demarcating a ventral respiratory and a dorsal alimentary zone. The groove areas fuse medially, separating the trachea from the oesophagus. At the caudal end of the tracheal portion the lung bud is developing, which later gives rise to paired pulmonary sacs or vesicles (Keibel and Mall, 1912). One theory holds that during this period cell buds may become separated off or sequestrated, later giving rise to the entodermal cysts under discussion. The aberrant cell groups are possibly of unspecified destiny or multipotential, since the ultimate cystic formation may be gastric, intestinal, or bronchial in structure.

Morbid anatomy and histology. The gross appearance of the cysts in previously reported cases has been varied. They may be small or large, simple or loculated, thin or thick walled. The majority have been large cysts on one side of the chest, nearly all on the right, and of these several have been much modified by multiple-stage operations before examination. Peptic ulcers have occurred in the intestinal cysts with perforation into the lung and haemoptysis. The contents of the cyst have been commented on by several authors but seldom fully examined. The fluid has been described as viscid, opalescent and milky. In Mixter and Clifford's (1929) first case the fluid was acid, and Nicholls (1940) reported in his case excoriation of the skin round the sinus following drainage of the cyst as an empyema. In the first case reported here the fluid was not examined chemically, but at the time of operation it had a spangled appearance suggesting the presence of cholesterol crystals.

Microscopically sections of individual cysts have been reported to show in a high degree of differentiation all the structures normally found in oesophageal, gastric, pyloric, intestinal and bronchial viscera. In a few instances two types of mucosa have been found in the same cyst, such as gastric mucosa and ciliated respiratory epithelium. The posterior mediastinal cyst recorded by Smith (1930), besides plain muscle in places in two layers showed both ciliated stratified (respiratory) epithelium and columnar epithelium with oxyntic cells (gastric). That described by Guillery (1937) was lined by epithelium both of tracheal and oesophageal types. The association of intrathoracic and abdominal cysts lined by gastric or intestinal mucosa has been recorded in several cases.

Symptomatology. Most of the cases of intestinal and bronchial cyst have occurred in infancy, and the symptoms have been mainly respiratory, but in some no respiratory disturbance was noticed by the parents. Mistaken diagnoses of pneumonia or pleural effusion have been reported in several instances. Respiratory function may be affected in several ways. A small cyst may occlude a bronchus and cause pulmonary collapse or, if occlusion is partial, obstructive emphysema as in Mixter and Clifford's third case, or such a cyst may compress the trachea as in the second case reported here. A large cyst will cause dyspnoea simply by its bulk without necessarily compressing a bronchus, and this type is likely to be mistaken for pleural effusion. In some of the cases pneumonia and empyema have been diagnosed, but no clear example of true infection of the cyst as occurs in cysts of the lung has been found. Pneumonia is a common misdiagnosis for collapse, and the contents of a cyst might be mistaken for the pus of empyema. That

gastric cysts may give rise to pleuritis is not surprising, as two of the cases collected by Nicholls had peptic ulcers in the wall of the cyst. In the first case reported here paracentesis yielded bloody fluid which presumably came from outside the cyst, although at operation the pleural cavity appeared clear.

Diagnosis. Without operation diagnosis cannot be made with any certainty except when aspiration of a gastric type of cyst produces acid fluid. In other cases aspiration may give no positive evidence and for small cysts may be impracticable. Radiography may show either a space-occupying lesion or its effect on the bronchus in producing collapse or obstructive emphysema. Good lateral films are essential, and unless the cyst is large will show its posterior position which distinguishes it from dermoids and cysts of pericardial origin. A large cyst may be difficult to distinguish from a pleural effusion unless (as in the first case) it has a rounded contour in the costophrenic sulcus. Bronchoscopy is of little help when the cyst is large, but in the case of small cysts the clinical picture may resemble that of foreign body or tuberculous adenitis, and here bronchoscopy is essential. The Mantoux test may also be of help if tuberculous adenitis is to be considered. Lipiodol bronchography will add no useful information unless bronchiectasis has supervened, but lipiodol injection into the cyst may be useful to demonstrate its shape and size. It seems impossible to distinguish between a large posterior mediastinal cyst and a ganglioneuroma. Erosion of bone, although suggestive of ganglioneuroma, may be caused by a cyst as in one of Mixter and Clifford's cases. In an infant of three months Guillery found at post-mortem one cyst of tracheal and oesophageal structure in the posterior mediastinum and another smaller cyst within the vertebral canal at the level of the fifth dorsal body. Symptoms of spinal compression are not mentioned, but had the child lived a dumb-bell ganglioneuroma would probably have been suspected. Paterson and Pilcher (1941) record a case of a dumb-bell ganglioneuroma in a child with a large right upper intrathoracic portion but only a small prolongation into a contiguous intervertebral foramen without pressure involvement of the cord.

Case reports

Case 1. Cyst of gastric structure. Female infant, aged twelve months, weighing 17 lb., born at full term, normal labour, with birth weight 6¼ lb. From the sixth month the mother thought the infant's abdomen was unduly large, and she found the child took its feeds badly and gained little after the seventh month. There was no history of cough or infection.

At twelve months the infant was admitted to the Hospital for Sick Children, Great Ormond Street, and on examination of the abdomen the edge of the liver was felt three fingerbreadths below the costal margin; the spleen was palpable on inspiration; there was no ascites and no tenderness.

The chest was pigeon-breasted with recession of the lower intercostal spaces on inspiration; the right half of the chest was markedly dull all over, with absence of breath sounds and of râles. The heart was displaced to the left with the apex beat in the axillary line. The respiration rate was within normal limits (25 to 40), the temperature normal, and the general condition of the infant fair.

An x-ray showed displacement of the trachea and heart to the left, and a uniform opacity of all of the right half of the thorax, except for a small clear area in the costo-phrenic angle, bordered on the third side by the convex edge of the opaque shadow. The lateral film in this case showed less opacity in the posterior part of the chest than was expected and no outline of the tumour could be seen. A provisional diagnosis of teratoma or of ganglioneuroma was made.

By bronchoscopy (Mr. J. Crooks, October 6, 1941), under ether and chloroform, the right bronchus was difficult to find and appeared flattened, the left normal; there was no excess secretion in either.

On paracentesis 10 c.c. of blood-stained fluid was easily obtained from the right chest, and the needle on being advanced impinged against a hard mass. The fluid deposit showed numerous serosal cells and some 'fat' phagocytes. Five days after aspiration the chest was screened and both sides of the diaphragm were seen to move well though the right half was at a lower level than the left; the right costo-phrenic angle was clear. On deep inspiration a rounded shadow was noticeable in the right lung field unattached to the chest wall laterally or to the diaphragm. Further x-rays were taken for evidence of bone erosion but none was found.

October 16, 1941. Operation by R.S.P. Anaesthetic: intratracheal gas, oxygen, ether by Miss Byrde. The chest was opened by a long intercostal incision on the right side. The pleural cavity was opened anteriorly and it was found that the right side of the chest was almost entirely filled by a cystic swelling with a thick vascular wall. The collapsed right lung could be seen in front of and below the swelling. The cyst was extrapleural, what was left of the pleural cavity lying anterior to it. The cyst seemed to have its main attachment in the paravertebral gutter. To facilitate manipulation the cyst was aspirated, 8 oz. of pale yellow spangled fluid being removed. The pleura over the cyst was incised and a plane of cleavage between it and the true wall of the cyst was easily found. This plane of cleavage was extended by blunt dissection and the whole cyst was removed by an extrapleural dissection. Separation at the lower pole and from the mediastinal structures was easy, but the cyst was more adherent in the paravertebral gutter at the apex of the chest and it was from this part that it appeared to derive its blood supply. After removal of the cyst there was left a large extrapleural cavity with a wide opening into the pleura where the latter had been incised. The pleura was stitched back to the chest wall to prevent it flapping across the chest. The chest was closed without drainage. The child stood the operation well and was transferred to an oxygen tent directly after operation. The post-operative course gave rise to no anxiety. Oxygen therapy was continued until the second morning after operation. X-ray on the second day showed the right lung beginning

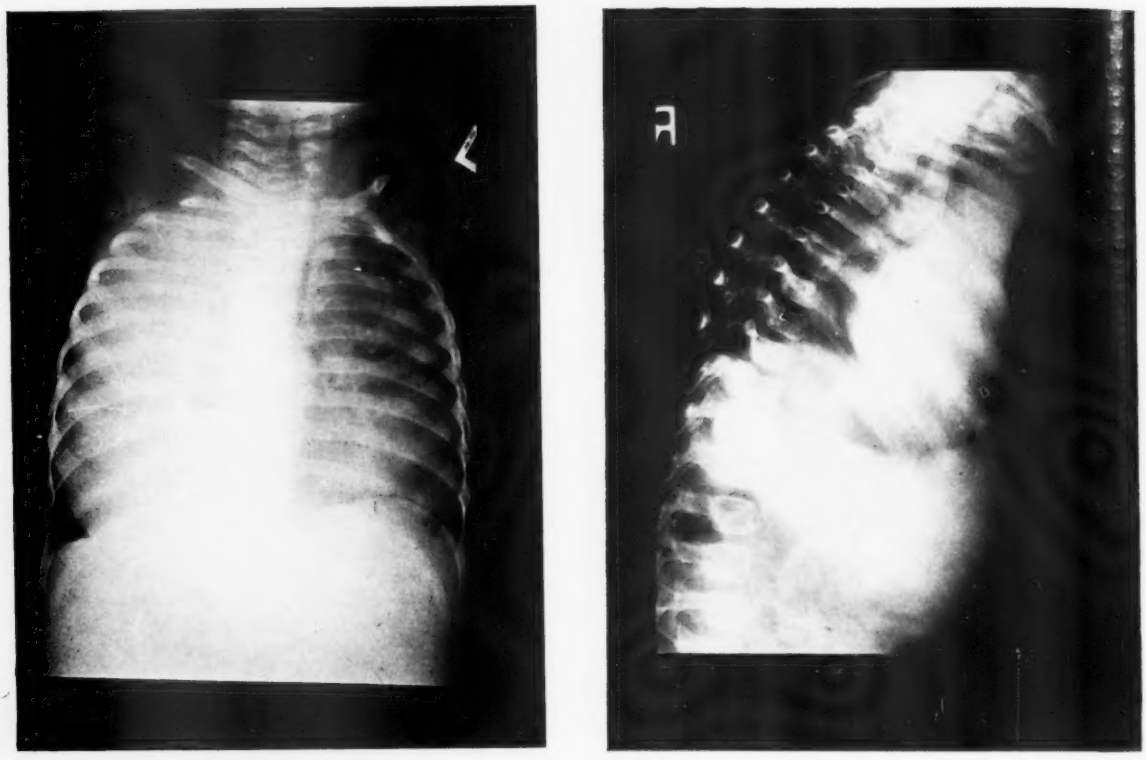

FIG. 1 and 2.—Case 1. Pre-operative x-rays showing cyst occupying right side of chest and displacing mediastinum to the left. The edge of the cyst can be seen outlining a small translucent area in the costophrenic angle.

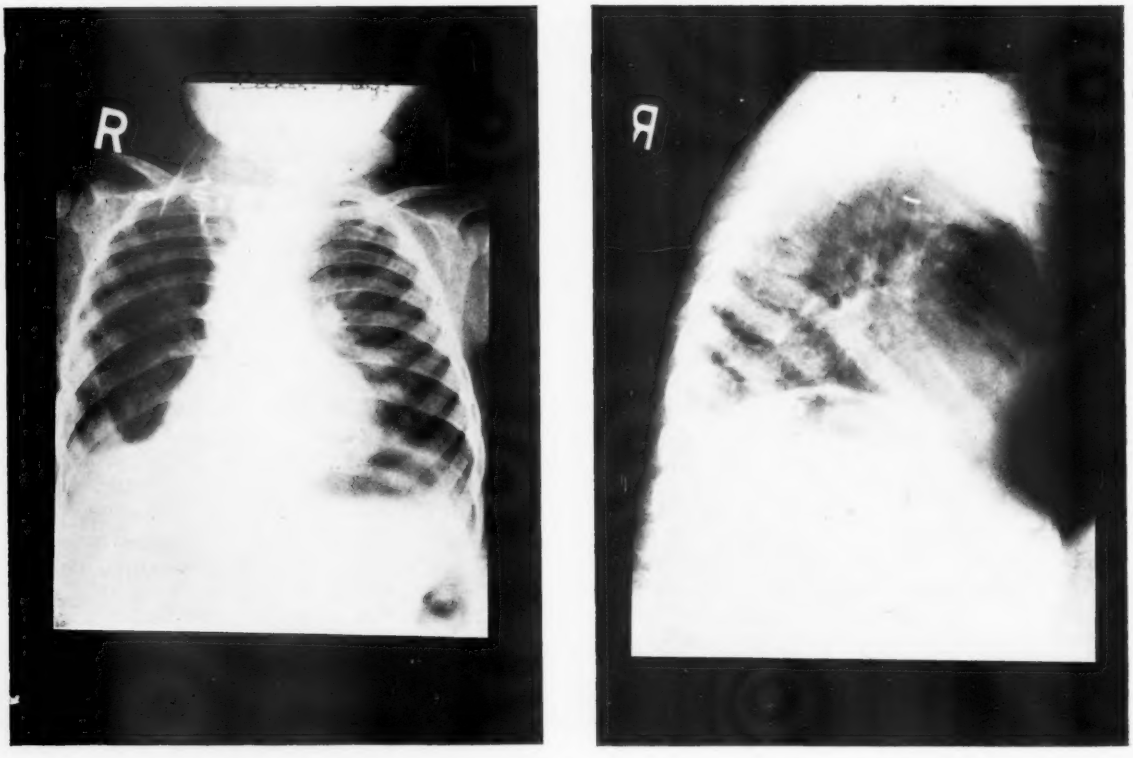

FIG. 3 and 4.—Case 1. Post-operative x-rays showing right lung expanded.

to expand but the mediastinum still displaced to the left. There was very little effusion in the pleura. X-ray a fortnight after operation showed that the right lung had re-expanded. After operation the child had signs of sympathetic paralysis of the right upper limb and right side of the face. This was not present before operation and no doubt the sympathetic was damaged during liberation of the upper part of the cyst.

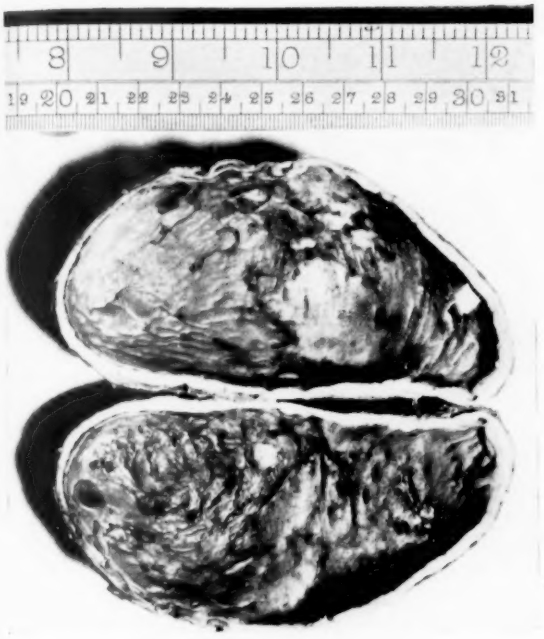

FIG. 5.—Case 1. Cyst opened longitudinally.

The cyst, diminished in size by aspiration, had a wall varying in thickness from 1 to 5 mm., its outer surface was shaggy and its inner surface lined with what looked like mucous membrane. Section of the wall of the cyst closely resembled that of a normal stomach with typical gastric mucous membrane and three layers of muscle fibres.

Case 2. Cyst of bronchial structure. Female infant, aged eighteen months, weighing 27 lb., progressed satisfactorily up to the sixth month when she had a sudden attack of wheezing, accompanied by a spasmodic cough for seven days. She was free from symptoms for the next two months, when she had a similar dyspnoeic attack, since when she had not been free from wheezing and a brassy cough. For the past nine months the symptoms had not progressed. There was no family history of allergy or of tuberculosis.

At eighteen months the infant was admitted to the Hospital for Sick Children, Great Ormond Street (November 25, 1941). She was active, cheerful, but had an almost constant wheeze or stridor: respirations were not usually increased (25 to 30). There were frequent bouts of coughing with a brassy character, as if something were choking her, in which she became much distressed and breathed rapidly. On examination of the chest nothing abnormal was found except recession of the lower ribs and intercostal spaces. After her bouts of coughing the wheezing was more noticeable for a time and catarrhal sounds could be heard in the chest. It was noted that the child held her chin down and resented any attempt to extend the neck.

Mantoux tests, 1 in 10,000 and 1000, were negative. Bronchoscopy (November 27, 1941) by Mr. Crooks, showed the first part of the trachea

FIG. 6 and 7.—Case 1. Microphotographs of cyst wall showing gastric type of mucous membrane and three layers of plain muscle.

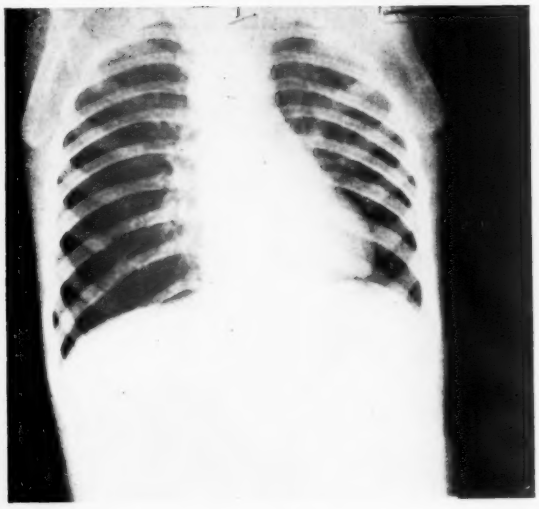

FIG. 8 and 9.—Case 2. Pre-operative x-rays: In the antero-posterior, the cyst produces a slight bulge to the right. In the lateral, forward displacement of the trachea can be seen.

was normal; but the second part, just above the bifurcation, narrowed down to a transverse slit. This appeared to be due to a diffuse swelling of the posterior wall which was patchy and red. Some deficiency of the cartilage rings was suspected at this level. The right and left bronchi were difficult to find but appeared normal. Oesophagoscopy, November 29, 1941 (Mr. Crooks), showed nothing abnormal except some deviation of the oesophagus to the right. An x-ray with 'opaque swallow' showed no evidence of obstruction. In a lateral x-ray and screening of the chest the trachea was seen to be displaced markedly forward and compressed, curving down over a well-defined mass of uniform opacity, while the oesophagus was displaced backwards and to the right. Perpendicularly the mass lay over the second to sixth dorsal vertebrae. No lesion of cervical or dorsal vertebrae was visible. A provisional diagnosis of post-mediastinal tumour, possibly ganglioneuroma, was made, and it was decided that the chest should be explored.

December 16, 1941. Operation by R.S.P. Anaesthetic: intra-tracheal gas, oxygen, ether by Miss Byrde. The chest was opened at the posterior end of the fourth space, a short piece of the posterior end of the fourth rib being resected. The pleura was stripped off the apex of the chest so that the mediastinum could be approached extrapleurally; this was only partly successful and the pleura was torn close to the azygos vein. The oesophagus was identified in the superior mediastinum, being displaced to the right. It appeared larger than normal and inflated with each inspiration. At first no tumour could be found, but after separation of some areolar tissue in front of the oesophagus a smooth soft swelling was exposed to the left of it and behind the trachea. The exposed part had a bluish colour. The azygos vein was then divided in order to increase the exposure, but shortly after this the child's respirations failed, and in spite of artificial respiration the heart also stopped beating and would not respond to massage.

POST-MORTEM EXAMINATION showed both lungs were almost completely collapsed. The pleural cavities were not examined for pneumothorax and it was not discovered whether the left pleura had

FIG. 10.—Case 2. Post-mortem specimen showing cyst and its relations exposed from behind, the right bronchus has been cut across close to trachea and removed.

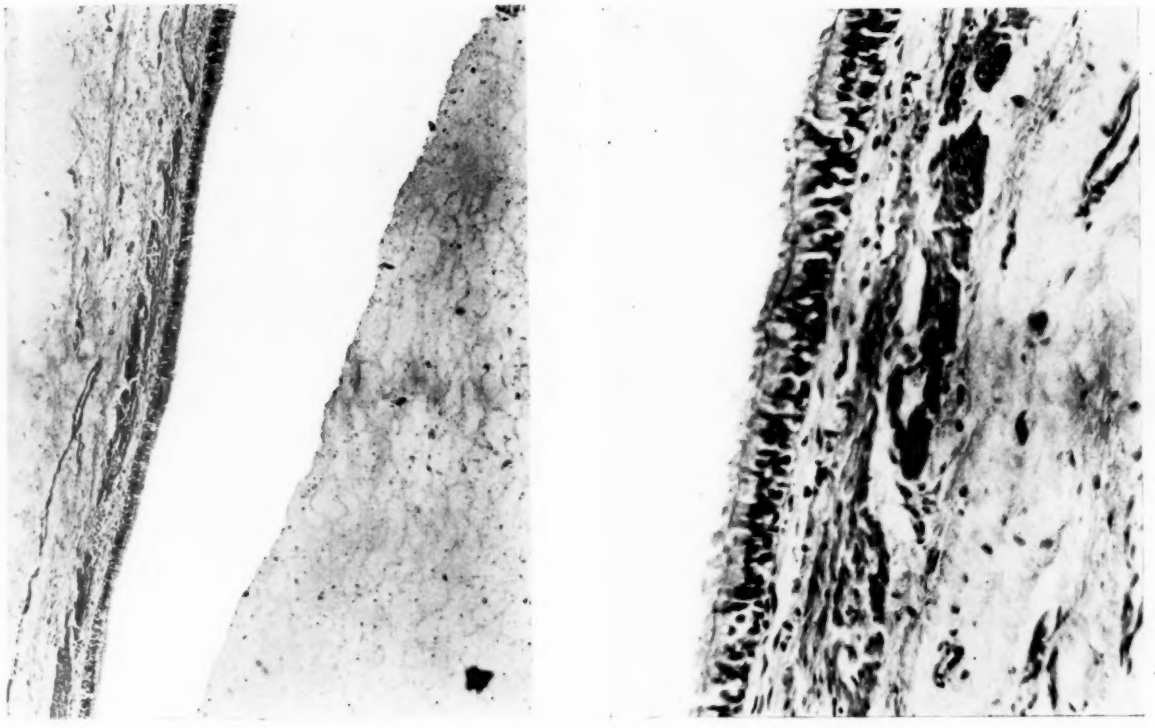

FIG. 11 and 12.—Case 2. Microphotographs of cyst showing epithelium of bronchial type and a single layer of plain muscle.

been torn during operation (this had been suspected as a possible cause of the child's collapse). Arising from the posterior aspect of the left bronchus at its origin was a cystic swelling, 3 cm. in diameter, which was globular in shape. There was a thin covering of mucous membrane over the cyst where it bulged into the trachea and left bronchus, and it looked as if very little violence would have ruptured it into the bronchus. There was some hypertrophy of the left ventricle and it was thought that the cyst might also have pressed on the arch of the aorta. Section of the wall of the cyst showed respiratory epithelium with a thin layer of plain muscle external to it.

Comment

These two cysts, although probably of similar origin, presented strikingly different clinical pictures and therapeutic problems. In neither case was a pre-operative diagnosis made except in so far as a benign condition amenable to surgery was suspected. The first cyst was large and produced symptoms by occupying space in the chest. There may have been some compression of the right bronchus, but it was not demonstrated and the radiographs suggested that there was some air-containing lung in front. At operation no intimate connexion with other structures in the chest was found and removal in one stage was not technically difficult. The rapid re-expansion of the lung was gratifying, for in such a case if the bronchus were blocked bronchiectasis might occur. It is worth noting that at first the cyst was regarded as a malignant neoplasm and the prognosis hopeless, a mistake which should not occur if the rarer benign conditions such as cysts and nerve tumours are kept in mind. In the second case the cyst was relatively small, but because of its situation and intimate relation to the large air passages, its effects were more serious. In spite of bronchoscopy it was not appreciated that the cyst was in the wall of the left bronchus nor was there any pre-operative suspicion of the collapsed state of the left lung found at post-mortem. This was assumed to be due to bronchial occlusion although, unfortunately, pneumothorax was not excluded. The problem of how to treat such a cyst is not easy. Exploration was undertaken in the hope of removing it completely, but post-mortem examination showed that this would have been difficult and would probably have necessitated opening the left bronchus. Such a difficulty might have been anticipated if more notice had been taken of the suspected deficiency of the tracheal rings. It is worth considering whether such a cyst should be dealt with through the bronchus. The removal of a portion of the cyst with punch forceps or diathermy should be possible although attended by a risk of infection. No record of successful treatment of a cyst of this type has been found, although several other large right-sided cysts have been successfully removed in stages.

Summary

1. Cysts of bronchial or intestinal structure or both occur in the posterior mediastinum.

2. They differ from dermoids in their posterior position and the early age at which they cause symptoms.
3. Symptoms may be due to a large space-occupying lesion or to bronchial obstruction.
4. Two cases, one of a gastric cyst and the other of a bronchial cyst, are described.
5. The gastric cyst was successfully removed in one stage by thoracotomy.
6. An attempt to remove the bronchial cyst by thoracotomy was fatal and the method was probably unsuitable.
7. It is suggested that the latter type of cyst should be attacked through the bronchus.

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CASE REPORTS

A CASE OF JUVENILE XANTHOMATOSIS

WITH ENLARGED LIVER AND SPLEEN AND GREATLY INCREASED PLASMA LIPOIDS

BY

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The present report describes an unusual syndrome in a girl aged seven years. The outstanding features of the case are extensive cutaneous xanthomatosis, enlargement of the liver and spleen, arrested growth and enormous increases in phospholipin, cholesterol, and cholesterol esters in the blood plasma, with little or no glycerol fat.

Case report

J. M., a girl aged seven years, came under the care of Dr. A. G. Ogilvie at the Royal Victoria Infirmary, Newcastle-upon-Tyne, in May, 1938. There was nothing of importance in the family history and there was no Jewish strain in the ancestry. At the age of two weeks the child had had a severe umbilical haemorrhage, and shortly afterwards developed jaundice. Repeated attacks of jaundice associated with bronchitis, continued up to the time when she came under observation, and had been more frequent during the period 1936–38. The skin lesions appeared in 1937, first on the fingers and elbows, and later increased in size and number and became generalized.

Examination showed an undersized child, but of normal proportions. (Weight 31 lb. 6 oz.) She was mentally bright and her general condition fairly good. At this stage there was only a slight icteric tinge in the conjunctivae, and no frank clinical jaundice. There was extensive cutaneous xanthoma. The hands were the most affected, showing raised yellow nodules over the dorsal surfaces of the knuckles and on the palms, and flat linear yellow streaks along the flexures in the palmar surfaces of hands and fingers (fig. 1). In the region of both elbows there were aggregations of raised nodules over the olecranon process and flatter streaks along the skin folds on the flexor surface. On both feet, nodules were present over the ball of the big toe and the heel. Small patches occurred around the knees, over the upper and outer edge of the patella on both sides, and in the popliteal fossae. There were none on the front of the trunk, a few over the scapulae and one on the vulva. The nodules were well marked over the face and neck, appearing on the back of the neck, in the skin flexures over the front of the neck, and at the corners of the mouth and eyes; there were large deposits in both ears. The child was constantly scratching.

FIG. 1

The throat was normal; the teeth were badly formed and carious.

The systolic blood pressure was 140 mm. Hg

but otherwise the cardio-vascular system was normal.

The liver was enlarged two fingerbreadths below the costal margin and the spleen also moderately enlarged.

There were no abnormal physical signs in the central nervous system, but while in hospital the child showed occasional choreiform movements, twitching of muscles on the outer side of thigh, and involuntary movements of flexion of the knees. The optic fundi were examined by Mr. J. S. Arkle; he found some choroid atrophy and a ' pepper and salt ' fundus, not regarded as of any special significance.

Radiological examination of the limbs, skull, pelvis, and chest showed no abnormality.

The Wassermann reaction was negative. The haematological examination showed: red cells, 3,580,000 per c.mm. (reticulocytes 2·6 per cent.), haemoglobin 65 per cent., colour index 0·91, leucocytes 8,600 per c.mm.

The urine showed heavy albuminuria and a gross excess of urobilin. Microscopic examination of the urinary deposit showed abundant leucocytes, some renal epithelial cells and a few ' fatty ' casts. Under the polarizing microscope, anisotropic spherocrystals were seen, some in casts and in epithelial cells, and some as free droplets.

A glucose tolerance test, with a dose of 1 gm. glucose per kgm. body weight, gave the following blood sugar curve: Before, 0·105 per cent.; ½ hr. after 0·140 per cent.; 1 hr. after, 0·122 per cent.; 1½ hr. after, 0·107 per cent.

The total serum bilirubin was 0·8 mgm. per 100 c.c. of which 0·32 mgm. per 100 c.c. gave the direct Van den Bergh reaction. The blood urea was 52 mgm. per 100 c.c. The fragility of the red blood cells was normal.

The plasma lipoids, in the fasting state, showed an extraordinary picture (see table 1). The lipoid phosphorus, estimated in the first instance as the total phosphorus in a Bloor extract, was 203 mgm. per 100 c.c., corresponding to 5080 mgm. per 100 c.c. as phospholipin. This amazing result was confirmed by separation of the phospholipins from the lipoid extract, as will be described more fully later. It appears to be unprecedented.

Sphingomyelin and glycerophosphatide (lecithin and kephalin) contributed about equally to the plasma phospholipins.

The total plasma cholesterol was 2600 mgm. per cent., of which 610 mgm. per cent. was free cholesterol. Such figures, though rare, have been recorded in other types of severe lipaemia. In this case, as in other pathological lipaemias, the increase in cholesterol and phospholipin was confined to the plasma. On May 20, 1938, the corpuscles were analysed and showed 403 mgm. per 100 c.c. phospholipin and 120 mgm. per 100 c.c. cholesterol.

The total fatty acids of the plasma were completely accounted for by fatty acids in combination as phospholipin and cholesterol ester. There was therefore no measurable amount of fat as glycerol ester.

A biopsy specimen of a cutaneous nodule was examined by Dr. J. G. Thomson. Paraffin sections

TABLE 1

PLASMA LIPOIDS OF J.M. IN THE FASTING STATE

Date	Regime	Plasma lipoids (mgm. per 100 c.c.)						Glyceride
		Total lipoids	Phospho-lipin	Total cholesterol	Free cholesterol	Cholesterol ester		
						Cholesterol	Fatty acid	
2.5.38	Full normal diet	9140	5080	2600	610	1990	1500	0
4.5.38		—	5270	—	—	—	—	—
20.5.38		6510 *	3970	1816	520	1296	613	0
June to October, 1938.	Light diet poor in fat							
20.10.38		3890 *	2185	979	521	458	364	227
23.10.38	Vegetable diet started							
8.11.38		—	1588	694	—	—	—	—
17.11.38		—	1880	750	—	—	—	—
24.11.38		—	1600	872	—	—	—	—
1.12.38		—	1860	757	—	—	—	—
1939	Light diet poor in fat							
10.2.40		5410	3350	1541	897	644	518	0
28.4.41		—	1783	1035	818	217	—	—

* The total lipoid on these occasions included a little unsaponifiable matter other than cholesterol. This accounts for the fact that total lipoids are a little higher than the sum of phospholipin, free cholesterol, cholesterol ester, and fat.

showed the characteristic accumulation of xanthoma cells, with no inflammatory reaction. Frozen sections examined under the polarizing microscope showed the spherocrystals characteristic of cholesterol ester.

The child remained in hospital through May, 1938. Before her admission the diet had included much milk. On a diet of lower fat content all fractions of the plasma lipoids, though still extremely high, showed some reduction from the initial level. After this investigation in hospital the child was sent home and during the next five months was taking a diet low in animal fat. This was not a strictly quantitative diet, but the intake of animal fat was probably about 10 gm. per day. During this period she was generally better in health, free from jaundice, and free from itching of the skin.

She was admitted to hospital again in October, 1938, in order to try the effect of a diet entirely free of animal fat as recommended by Thannhauser (1935).

At this stage the liver and spleen were still enlarged and the xanthoma was just as extensive as in May. Her weight was now 33 lb. 10 oz. On admission a laevulose tolerance test was made after 15 gm. laevulose, with analysis of the blood laevulose by the writer's method (Herbert and Davison, 1938). The rises in blood laevulose above the fasting level were: at ½ hr., 14·5 mgm. per 100 c.c.; at 1 hr, 13·0 mgm. per 100 c.c.; and at 2 hr., 12·0 mgm. per 100 c.c. This curve falls near the upper limit of normal, and shows no definite deficiency in laevulose tolerance (Herbert and Davison, 1938; Herbert, 1939).

The total serum bilirubin was at this time 0·56 mgm. per 100 c.c. and there was a positive direct Van den Bergh reaction. The blood urea was 39 mgm. per 100 c.c. The urine still contained albumin but no other abnormal constituent, and the deposit at this time was normal.

As a result of a suggestion made by Dr. Thannhauser in correspondence about the case, a test of the response of the blood sugar to adrenaline was made. It showed a normal rise of 0·038 per cent. above the fasting level, which was 0·08 per cent.

The plasma lipoids on admission were still very high, though the levels had fallen considerably from those observed in May (table 1).

A diet consisting entirely of vegetable foods was next instituted. The rationale of this treatment is that vegetable sterols are not absorbed, so that by a purely vegetable diet all exogenous cholesterol is eliminated. The daily diet gave 1430 calories (94 calories per kgm. body weight), 42 gm. protein (2·75 gm. per kg. body weight), 206 gm. carbohydrate and 41 gm. fat. The principal sources of protein were cereals (bread and oatmeal), lentils, and peanuts, and the fat was obtained chiefly from vegetable margarine and peanuts. Calcium and vitamin D were given as supplements. The only fluids given were water and lemonade. The child took the diet well and it was maintained for six weeks.

After a fortnight on this regime the plasma phospholipin had fallen from 2180 mgm. per 100 c.c. to 1590 mgm. per 100 c.c., and the total cholesterol from 979 mgm. per 100 c.c. to 694 mgm. per 100 c.c.; after this the levels fluctuated but fell no further.

This response to treatment was considered insufficient to justify the maintenance of a difficult quantitative diet, and the child was discharged on the less stringent diet which she had previously taken at home. This was continued until February, 1940, when she came into hospital again for investigation.

Her age was now nine years and her weight 35 lb. The xanthoma showed the same distribution as before, but the lesions were larger. The liver and spleen remained enlarged but were rather smaller than before (liver one fingerbreadth below the costal margin, and the spleen palpable only on inspiration).

The albuminuria was still present, and the urine deposit showed numerous leucocytes, and a few renal epithelial cells, but no casts.

The total serum bilirubin was 1 mgm. per cent. and there was a faint direct Van den Bergh reaction. The blood urea was 50 mgm. per 100 c.c.

The laevulose tolerance test was repeated, and the rises in blood laevulose above the initial level were 5·7, 5·7, and 0 mgm. per 100 c.c. at ½, 1, and 2 hr. respectively.

The plasma lipoids had risen during the period at home, but were not so high as when the child first came under observation. The characteristics of the lipoidaemia were the same as before; there was the same preponderance of phospholipin, with great rises also in free and combined cholesterol and absence of glycerol fat.

In April, 1941, the child had another spell in hospital. Her weight was now 39 lb. and her height 3 ft. 7½ in. The xanthomatous lesions were as extensive as before and similarly distributed. In addition, lesions were noticed on the gums. The liver extended one fingerbreadth below the costal margin and the spleen was just palpable. The heart was enlarged and the blood pressure had increased to 165 mm. systolic, 100 mm. diastolic.

The eyes were examined again by Mr. Arkle, who reported an exaggerated form of 'tiger-striped' fundus.

The blood count showed: red cells, 3,590,000 per c.mm.; haemoglobin, 70 per cent.; colour index, 0·97.

In the laevulose tolerance test the rise of blood laevulose above the apparent fasting level was at ½ hr. after the test dose, 10·9 mgm. per 100 c.c.; at 1 hr., 11·9 mgm. per 100 c.c.; and at 2 hr., 4·4 mgm. per 100 c.c.

The serum bilirubin was now normal (0·32 mgm. per cent.) and the direct Van den Bergh reaction was negative. The ordinary level of blood urea was 56 mgm. per 100 c.c.; it rose to 140 mgm. per 100 c.c. 1½ hr. after a dose of 10 gm. urea. In the urea concentration test the maximum concentration of urea in the urine was 1·46 per 100 c.c. The standard blood urea clearance (corrected for body size) was 26 per cent. of normal.

The urine still contained a large amount of albumin and the deposit showed numerous leucocytes, occasional red blood cells, and some renal epithelial cells, but no casts. A catheter specimen gave a growth of B. coli.

The plasma lipoids remained at a level greatly above normal, though lower than they had been fourteen months earlier (see table 1).

At the end of March, 1942, the child's medical attendant reported that she was active and in good general condition, but had not grown.

The plasma lipoids

Analytical schemes. The volume of plasma used for the analysis on May 2, 1938, was 4 c.c. and for the later full analyses 6·5 to 7 c.c. The amounts of lipoid present were so large that, even with these amounts of plasma, it was possible to employ methods depending on the separation and gravimetric estimation of the various fractions.* An analytical scheme suitable for lipaemic plasma has already been described (Herbert, 1937). The details will not be repeated here, but the scheme provided for analysis of three samples of a primary lipoid extract, one being used for estimation of lipoid phosphorus, one for the gravimetric estimation of free cholesterol, and the third, and largest, sample for the saponification of the total lipoids and the separation and gravimetric estimation of the total cholesterol and total fatty acid. The fat (triglyceride) was calculated indirectly from the fatty acid. The total fatty acid includes fatty acid originally in combination as phospholipin, cholesterol ester, and fat. Since phospholipin and combined cholesterol were known, the fatty acids in these fractions could be allowed for and the remaining fatty acid not thus accounted for was taken as the fatty acid combined as fat.

The first sample analysed in the investigation of the present case was dealt with by essentially the same scheme as the above, but as soon as the special features of the case were known, a different scheme was adopted in which the phospholipins were precipitated with acetone and magnesium chloride and the acetone-insoluble and acetone-soluble fractions separately worked up. This allowed of the separate investigation of the phospholipin fraction; it also gave better conditions for the calculation of the glycerol fat fraction, as will be explained later.

The new scheme of analysis, in its most complete form, is summarized in table 2. The principal difference from the old scheme is in the separation of the acetone-soluble and acetone-insoluble lipoids. (When an estimate of the total acetone-insoluble lipoids was required, it was obtained from the difference between the total ether-soluble lipoids and the acetone-soluble lipoids, as indicated in the table; it was only for this purpose that the weights of these extracts were needed, and they were not taken on every occasion.) There are two other differences from the earlier published scheme. The first is the removal of the alcohol and ether from the original Bloor extract at a temperature below 40° C. in order to avoid loss of phospholipin (Herbert, 1937; Ellis and Maynard, 1937). The second difference is that in dealing with the acetone-soluble fraction, the digitonide precipitation of the free cholesterol is first performed, and the lipoids not precipitated (cholesterol ester and fat) are collected quantitatively and used for saponification and separation of unsaponifiable matter and fatty acids. This was done for the sake of economy of material.

The phospholipin precipitate. Observations were

* For example, in the specimen of May 2, 1938, a sample of the lipoid extract corresponding to 3 c.c. plasma yielded 78 mgm. of white crystalline unsaponifiable matter, which on analysis was found to be entirely cholesterol.

TABLE 2

PRIMARY EXTRACTION OF PLASMA LIPOIDS BY BLOOR'S METHOD (7 c.c. PLASMA TO 250 c.c. TOTAL VOLUME)

Sample for phosphorus

MAIN FRACTIONATION (200–225 c.c. Bloor extract)
1. Solvent removed in vacuo below 40° C. Residue extracted with ether.
2. Solvent removed from ether extract. Weight of total lipoids obtained.
3. Lipoids re-dissolved in ether. Phospholipins precipitated with acetone and $MgCl_2$. Quantitative separation of acetone-insoluble and acetone-soluble fractions.

PHOSPHOLIPIN PRECIPITATE

Weight calculated by difference between total and acetone-soluble lipoids

Precipitate used for one or more of the following analyses:

1. Phosphorus
2. Phospholipin fatty acids
3. Sphingomyelin by reineckate method

ACETONE SOLUBLE FRACTION
1. The lipoids were freed from the $MgCl_2$ as follows: Acetone solution diluted with water. Lipoids extracted with mixed ether and petroleum. Extract washed with water.
2. Solvent removed and lipoids weighed.
3. Lipoids dissolved in alcohol and treated with digitonin for precipitation of free cholesterol.

DIGITONIDE PRECIPITATE

Gravimetric estimation of free cholesterol

LIPOIDS NOT PRECIPITATED BY DIGITONIN. Collected quantitatively in ether solution, and saponified with sodium ethylate. Unsaponifiable matter and fatty acids separated and purified.

UNSAPONIFIABLE FRACTION
1. Weight of unsaponifiable matter obtained
2. Estimation of cholesterol

FATTY ACIDS

Weight obtained

made on the precipitated phospholipins firstly, to confirm the extraordinarily high figures obtained in estimations of lipoid phosphorus, and secondly, to get information on the proportions of glycerophosphatides and sphingomyelin in the phospholipin mixture.

On two occasions, the total acetone-insoluble lipoids were estimated, as well as the phosphorus in the phospholipin precipitate. On May 20, 1938, the total phospholipin, estimated from the acetone-insoluble lipoids, was 3970 mgm. per 100 c.c. plasma, and the phosphorus in the precipitate 141 mgm. per 100 c.c. plasma; on February 10, 1940, the total phospholipin precipitated was 3970 mgm. per 100 c.c. plasma, and the phosphorus 136 mgm. per 100 c.c. plasma. The phosphorus, expressed as a percentage of the phospholipin, was 3·55 per cent. and 3·43 per cent. on these occasions.

The proportion of phosphorus to total phospholipin varies somewhat for different phospholipins, but the following are theoretical values for some typical compounds: Palmityl-oleyl lecithin, 3·99 per cent.; stearyl-arachidonyl lecithin, 3·75 per cent.; stearyl-linolyl kephalin, 4·17 per cent.; stearyl arachidonyl kephalin, 4·04 per cent.; lignoceryl sphingomyelin, 3·72 per cent. It has been usual, in calculating phospholipin from lipoid phosphorus, to assume the factor 4 per cent. as the phosphorus content of phospholipin. Ellis and Maynard (1937) determined the factor for the phospholipins of bovine plasma and found 3·57 per cent., a value close to that obtained in the present work. Possible analytical errors in these determinations are the over-estimation of the total acetone-insoluble material due to slight contamination with non-lipoid material, and the under-estimation of the phosphorus of the precipitated phospholipin due to slight losses in manipulation. Both would have the effect of lowering the percentage of phosphorus found. For convenience the conventional value of 4 per cent. has been used in calculating phospholipin from phosphorus in the present work, and the figures given in table 1 were obtained in this way. Any error due to the use of this factor would result in figures slightly lower than the true values.

The behaviour of the phosphorus at different stages of the analytical scheme has also been followed. Comparisons between the phosphorus of the original Bloor extract, of the ether extract obtained from it (after removal of the solvent and extraction of the residue) and of the phospholipin precipitate are shown in table 3. The phosphorus of the Bloor extract is completely recovered in the ether extract, and there is either a slight loss, or none, during precipitation and re-solution of the phospholipin.

TABLE 3

Date	Lipoid phosphorus, mgm. per 100 c.c. plasma, found in:		
	Original Bloor extract	Ether extract	Phospholipin precipitate
4.5.38	211	208	189
20.5.38	157	154	141
20.10.38	87·7	—	87·4
10.2.40	134	—	136

These results confirm that the high phosphorus of the Bloor extract is all, or almost all, true lipoid phosphorus.

Plasma phospholipins consist of a mixture of glycerophosphatides (lecithins and kephalins) and sphingomyelins. The glycerophosphatides have one atom of phosphorus and two fatty acid radicles in the molecule, and the sphingomyelins one atom of phosphorus and one fatty acid radicle. The ratio of phosphorus to fatty acid can therefore be used as a measure of the proportion of glycerophosphatide and sphingomyelin.

Estimates of the phospholipin fatty acids were made on two occasions (May 4, 1938 and February 10, 1940). On the first occasion, the separated phospholipins were saponified with sodium ethylate, the fatty acids were liberated from the soaps obtained, purified, and estimated both by direct weighing and titration. The ratio found was one atom of phosphorus to 1·55 molecules of fatty acid. As the amount handled was small, this is an approximate figure. It corresponds to a mixture of 45 per cent. sphingomyelin and 55 per cent. glycerophosphatide. Further experience with this procedure showed that alkaline hydrolysis of phospholipin under these conditions might involve difficulties in quantitative handling, probably due to the presence of magnesium, as noted by Stewart and Hendry (1935). Therefore, on February 10, 1940, the phospholipins were subjected to acid hydrolysis. The phospholipins in methyl alcohol solution were hydrolysed with hydrochloric acid, the resulting methyl esters of the fatty acids were extracted, purified, and weighed, and the fatty acids calculated from the weight of the methyl esters. The ratio obtained was one atom of phosphorus to 1·61 molecules of fatty acid. This corresponds to a mixture of 39 per cent. sphingomyelin and 61 per cent. glycerophosphatide.

On another occasion (October 20, 1938) sphingomyelin was directly estimated by the method of Thannhauser and Setz (1936). The phospholipin precipitate was dissolved in methyl alcohol and the sphingomyelin precipitated as the reineckate and estimated gravimetrically.* The sphingomyelin amounted to 692 mgm. per 100 c.c. plasma, and the total phospholipin 2185 mgm. per 100 c.c. plasma; or, of the phospholipin mixture, 32 per cent. was sphingomyelin and 68 per cent. glycerophosphatide.

These results show that both the glycerophosphatide fraction and the sphingomyelin are enormously increased in this case, and that the proportions of the two fractions are not greatly different from those occurring normally. Stewart and Hendry (1935) found that for the phospholipins of whole blood the ratio of phosphorus to fatty acids was one atom of phosphorus to 1·5 molecules of fatty acid, indicating equal parts of glycerophosphatide and sphingomyelin. Thannhauser and Setz (1936), using the reineckate method, found varying proportions in normal human plasma, the sphingomyelin usually constituting from 44 to 64

* In calculating the sphingomyelin from the weight of its reineckate the factor of Thannhauser and Setz has been used (sphingomyelin =reineckate ×0·877). The sphingomyelin complex in serum gives two reineckates, one soluble and the other insoluble in cold acetone. The acetone-insoluble fraction is regarded by the authors as a reineckate of a trimeric sphingomyelin, and it is to this compound that the factor 0·877 applies. The other reineckate may be formed from a monomeric sphingomyelin. The effect of using the factor 0·877 for the total reineckate, as has been done in the present work, will probably be a slight overestimate of the sphingomyelin.

per cent. of the total phospholipin, with occasional higher values.

The calculation of fat (glyceride). In the older scheme of analysis (Herbert, 1937) the total fatty acids of plasma were estimated. The fatty acids of phospholipin and cholesterol ester were calculated, and the remainder of the fatty acid not accounted for as phospholipin and cholesterol ester represented the fatty acid in combination as glycerol fat. This method involves an uncertain assumption as to the fatty acid obtained from phospholipin, since the fatty acid corresponding to the total phospholipin varies according to the proportions of glycerophosphatide and sphingomyelin in the mixture. No serious error is introduced when phospholipin forms a small proportion of the total lipoids, but in the present case, with the large proportion of phospholipin, the fat could only be calculated by the above method if the proportion of fatty acid in the phospholipin mixture was known.

In the analysis of May 2, 1938, in which the acetone-insoluble and acetone-soluble lipoids were not separated, the calculation was made on the basis of the determination of phospholipin fatty acids two days later. The total fatty acids were completely accounted for as phospholipin and cholesterol ester.

In the analyses of May 20, 1938, October 20, 1938, and February 10, 1940, the acetone-insoluble and acetone-soluble fractions were separated. The fatty acids of the acetone-soluble lipoids can only be derived from cholesterol ester and fat, so that if fat is present, there will be an excess of fatty acids relative to combined cholesterol. On May 20, 1938, the amount of fatty acid obtained was actually less than would have been expected from the combined cholesterol. It was shown that this was not due to incomplete saponification. It might be explained if some of the cholesterol was in combination with short-chain fatty acids which would be lost during the washing of the fatty acids. On October 20, 1938, there was a definite fat fraction, though it was a small amount compared with the other lipoids. On February 10, 1940, the fatty acid of the acetone-soluble fraction was exactly equivalent to the combined cholesterol.

Discussion

The mechanisms involved in the metabolism and transport of lipoids, and the physiological relations between the different lipoids, are by no means fully understood, and it is therefore impossible to give an adequate account of the derangements which lead to abnormal accumulations of lipoid in the blood and tissues in pathological conditions. In general, rises in blood lipoids may occur as a result of excessive mobilization, or diminished excretion, or over-production in endogenous metabolism. Abnormal accumulations of cholesterol in tissues (excluding purely local conditions) may be the result of gross increases in the plasma cholesterol, or may occur as a lipoidosis involving the reticulo-endothelial system without abnormalities in the blood.

The lipoidaemia of diabetes, and of renal disease (lipoid nephrosis and subacute nephritis) are probably due to increased mobilization of lipoids from the tissues. The hypercholesterolaemia of diabetes may be of extreme degree and may lead to cutaneous xanthoma and to deposits of cholesterol ester in the reticulo-endothelial system. The hypercholesterolaemia of the 'nephrotic syndrome,' though often considerable, does not ordinarily reach the levels met with in diabetic xanthomatosis, and does not lead to cutaneous xanthoma. Cholesterol ester is deposited in the kidneys, but the relation between the plasma lipoids and kidney deposits is obscure.

In biliary obstruction the plasma cholesterol is increased as a result of retention of cholesterol which would normally be excreted in the bile. The association of xanthomatosis with jaundice is well known, but, as will be indicated later, it is not possible to attribute this association to biliary obstruction alone.

There remain a number of conditions in which generalized xanthomatosis occurs, with or without hypercholesterolaemia, in which the primary cause is unknown, and which must be classed as 'idiopathic.' A classification of these conditions has been put forward by Thannhauser and Magendantz (1938). These authors regard all these types of 'primary essential xanthomatosis' as due to a cellular metabolic disorder of the reticulo-endothelial system with overproduction of cholesterol. The group of diseases is, however, heterogeneous, and may well include conditions of different etiology. The chief interest in the classification of Thannhauser and Magendantz is their analysis of the different conditions in relation to plasma cholesterol, the type of skin lesions found, and the liability of different internal organs to involvement in the lipoidosis. It is used here as an empirical classification without accepting the authors' etiological theory as applying throughout the group.

There are two main divisions, the 'hypercholesterolaemic' and the 'normocholesterolaemic' type.

In the 'hypercholesterolaemic' type the rises in plasma cholesterol are of varying degree but often are not very great, so the xanthomatosis is probably not simply the result of the high blood cholesterol. In this type the skin lesions have the form of 'xanthoma tuberosa' and 'xanthoma plana.' Associated lesions are xanthomata of tendons and tendon sheaths, and of the endocardium and blood vessels, and the syndrome of 'xanthomatous biliary cirrhosis' of the liver. The authors' analysis of cases seems to indicate two subdivisions in the hypercholesterolaemic group. In the first are grouped cases with tendon xanthoma, skin lesions, and cardiovascular lesions, occurring separately or together. In the second subdivision comes the syndrome of xanthomatous biliary cirrhosis with jaundice and skin lesions.

In 'primary essential xanthomatosis of the normocholesterolaemic type' the plasma cholesterol is normal; the skin lesions, when present, are of the form 'xanthoma disseminata,' and the

other organs which may be involved, separately or together, are the bones (Schüller-Christian syndrome), the pituitary, tuber cinereum, and other parts of the brain, and the lungs and pleurae.

Thannhauser and Magendantz regard ' primary essential xanthomatosis ' as analogous to the other primary lipoidoses, Niemann-Pick's disease and Gaucher's disease. All are conditions in which lipoids accumulate in the cells of the reticulo-endothelial system. In the xanthomatoses the lipoid is cholesterol ester. In Niemann-Pick's disease phospholipin, and to a less extent cholesterol, are involved (Epstein and Lorentz, 1930, 1932; Baumann, Klenk and Scheidegger, 1936; Sobotka et al., 1933; Tenuissen, 1937; Tropp and Eckardt, 1936, 1937), and it has been shown by Klenk (1934, 1935) that the phospholipin which accumulates in the tissues is sphingomyelin. There may be a rise in blood cholesterol, but when this occurs it is not usually very great (Baumann, Klenk and Scheidegger, 1936). The plasma phospholipin in Niemann-Pick's disease has not been adequately studied. In Gaucher's disease the lipoid is a cerebroside, kerasin, a compound of glucose, sphingosine and lignoceric acid, and this is found in the tissues without any accumulation in the plasma. This condition is excluded from the present discussion, which is concerned only with phospholipins and cholesterol.

In all the conditions in which plasma cholesterol and cholesterol esters are increased, there is also a rise in the plasma phospholipin. This is true of diabetic lipaemia, and the lipaemia of the nephrotic syndrome. Biliary obstruction also leads to increases in plasma phospholipin as well as cholesterol (Chanutin and Ludewig, 1936; Stroebe, 1935). In various types of xanthoma associated with hypercholesterolaemia, with or without jaundice, rises in phospholipin have also been recorded. (Buerger and Grutz, 1932; Chanutin and Ludewig, 1937; Herbert, 1937; Montgomery and Osterberg, 1938; Thannhauser and Magendantz, 1938). There is therefore some relation between phospholipin and cholesterol, common to all conditions in which cholesterol is increased in the plasma, in spite of the great variety in the etiology. Yet the lipoidaemia in the case here reported has special features. In most pathological lipaemias the greatest increase is in the fat; cholesterol and cholesterol esters come next in order, and the phospholipins show less increase (see Herbert, 1937, and other literature there cited). In the present case the order was reversed; there was little or no fat, an enormous rise in cholesterol and cholesterol ester, and an even greater rise in phospholipin.

In the case here reported, there was intermittent jaundice for many years, followed by the appearance of cutaneous xanthomatosis. The jaundice was slight or absent during the period of observation (1938 to 1941) when the features of the case were gross increases in plasma phospholipin, cholesterol, and cholesterol ester, cutaneous xanthoma taking the form of ' xanthoma tuberosa ' and ' xanthoma plana,' enlargement of the liver and spleen, and renal disease with azotaemia and hypertension. There was no oedema at any stage of the disease, and diabetes was excluded.

The rise in plasma lipoids was so extreme that it is reasonable to suppose that the lipoid deposits in the tissues were secondary to the lipoidaemia, but the cause of the lipoidaemia is quite obscure. Possible primary causes suggested by the clinical picture are renal disease and jaundice. The renal disease ran its course without oedema and with azotaemia and hypertension, so that the picture bore no resemblance to lipoid nephrosis. At one time the urine deposit showed spherocrystals under the polarizing microscope, indicating that the kidney was involved in the lipoidosis, but later examinations were negative. Moreover, the increases in plasma phospholipin and cholesterol were far greater than would be expected if the renal disease were the primary abnormality. It seems that the involvement of the kidney was incidental to the general disorder of lipoid metabolism rather than the primary condition.

The next question that arises is the association of jaundice and xanthoma. This association is well known, and in the majority of cases reported the jaundice has been present for months or years before the development of cutaneous xanthomatosis. Some authors have considered that biliary obstruction, with consequent retention of cholesterol, accounts for the syndrome. It is difficult to accept this interpretation, because chronic biliary obstruction is a common condition and does not usually lead to cutaneous xanthomatosis even when the obstruction is of long standing. In the case reported in the present paper, biliary obstruction cannot account for the syndrome, as there was no indication of extrahepatic biliary obstruction, and the lipoidaemia and xanthomatosis persisted for a long period after the jaundice had almost disappeared.

Thannhauser and Magendantz (1938) have drawn attention to the syndrome of ' xanthomatous biliary cirrhosis.' The clinical picture is of chronic enlargement of the liver and spleen with jaundice and hypercholesterolaemia, and cutaneous xanthoma. They believe that the primary abnormality is a disorder of cholesterol metabolism, leading to xanthomatosis of the larger bile ducts, followed by scarring and partial obstruction, which in turn initiates biliary cirrhosis of the liver. It certainly seems that a large proportion of the recorded cases of chronic jaundice with cutaneous xanthoma have shown biliary cirrhosis, but it is not easy to determine whether the xanthomatous diathesis precedes the liver disease or follows it. The chronic jaundice precedes the cutaneous lesions, and at the time when cutaneous xanthomata occur the hypercholesterolaemia is sometimes extreme. For example Dyke (1928) reported a case of this type with a total plasma cholesterol of 1250 mgm. per 100 c.c. and free cholesterol 1030 mgm. per 100 c.c. Chanutin and Ludewig (1937) recorded one with total plasma cholesterol ranging from 632 to 1480 mgm. per 100 c.c., with low

cholesterol ester, and with plasma phospholipins 1280 to 2480 mgm. per 100 c.c. (Figures re-calculated and expressed as phospholipin. In the original they are given as lipoid phosphorus.) The second case of Buerger and Grutz (1932) is similar, with total plasma cholesterol 2575 mgm. per 100 c.c., free cholesterol 1440 mgm. per 100 c.c., and phospholipin 3045 mgm. per cent., and Weidman and Boston (1937) described another case with blood cholesterol 1020 mgm. per 100 c.c. The syndrome may occur with more moderate degrees of hypercholesterolaemia, but on the whole it would seem that the skin lesions are secondary to the increase in blood lipoids, and it is very doubtful whether such cases should be classed as ' primary essential xanthomatosis ' in the sense in which Thannhauser and Magendantz use the term, as a disorder originating in the cells which become xanthoma cells.

It seems unlikely that the case reported in the present paper is one of xanthomatous biliary cirrhosis. The subsidence of the jaundice is strongly against the diagnosis of biliary cirrhosis. Also there was never any evidence of deficient hepatic function in the laevulose tolerance test based on analysis of blood laevulose, though this was repeated at intervals during three-and-a-half years. Another point against damage to the liver parenchyma is the high level of cholesterol ester in the plasma. Liver damage is characteristically associated with deficient formation of cholesterol ester and a decreased ratio of combined cholesterol to free cholesterol in the plasma. In the present case, the first observation showed that the cholesterol ester was very high, being increased in about the same proportion as the free cholesterol. Later, with diminution of the lipoidaemia, the cholesterol ester showed a greater fall than the free cholesterol and phospholipin, but still remained abnormally high in absolute amount. Therefore, although the possibility of an early cirrhosis cannot be excluded, the evidence is against any gross disorganization of the liver.

It is probable that the enlargement of the liver and spleen was due to lipoid deposits. So far as the period of investigation is concerned, the high blood lipoids would account for it. It is more difficult to interpret the jaundice of the preceding years, but it may have been due to early involvement of the liver in a lipoidosis which later became more generalized. It is of interest to consider other cases in which the liver was involved, but probably without cirrhosis.

Montgomery (1938) records the case of a girl aged nineteen years. She had an attack of jaundice at the age of ten, lasting three weeks, and immediately followed by cutaneous xanthoma tuberosum. The liver function was normal at the time when she came under observation, though the xanthomata were still present and the plasma cholesterol was 641 mgm. per 100 c.c.

Another case with involvement of the liver and spleen without jaundice is the first case of Buerger and Grutz (1932). This was a boy aged twelve. He had cutaneous xanthomatosis dating from the first year of life, and a history of ' disease of the spleen and nephritis ' at the age of eleven. The liver and spleen were enlarged. The analysis of the plasma at the first observation showed: phospholipin 1740 mgm. per 100 c.c., total cholesterol 686 mgm. per 100 c.c., free cholesterol 310 mgm. per 100 c.c., combined cholesterol 376 mgm. per 100 c.c., and total lipoids 9476 mgm. per 100 c.c. On a fat-free diet, the blood lipoids fell in a striking manner, though not quite to normal; the liver and spleen returned to normal size, and the xanthomatosis regressed, so that at the end of eight months only the remains of old lesions were to be seen.

This case of Buerger and Grutz is similar to that reported in the present paper. Both are instances of juvenile xanthoma with enlargement of the liver and spleen and with great increases in plasma phospholipin and cholesterol. The most important difference is in the response to a fat-free diet, which was good in the case of Buerger and Grutz and unsatisfactory in the present case.

This case, and others already mentioned, showed greatly increased plasma phospholipin as well as increased cholesterol. Not many authors have considered the plasma phospholipins in xanthomatosis, but wherever analyses have been made hypercholesterolaemia has been associated with high plasma phospholipin. In the work of Thannhauser and Magendantz (1938) and in the present study, rises in both sphingomyelin and glycerophosphatides have been found. The question therefore arises whether the primary metabolic disorder affects all these lipoids or whether their association in the blood is connected with the mechanisms regulating transport. It is noteworthy that in the present case, the two cases of Buerger and Grutz (1932), and the case of Chanutin and Ludewig (1937), all of which are instances of xanthomatous disease with involvement of the liver, the increases in plasma phospholipin are even more striking than the increases in cholesterol, high as these are. On the other hand in diabetic lipaemia with a comparable degree of hypercholesterolaemia the increase in phospholipin is relatively less (Herbert, 1935, 1937). Therefore it seems probable that the gross increase in plasma phospholipin found in some xanthomatous diseases is an expression of some fundamental disturbance of phospholipin metabolism, and not a secondary result of hypercholesterolaemia.

For this reason it is of interest to consider whether there is any relation between these cases of xanthoma and the Niemann-Pick syndrome. The typical picture of Niemann-Pick's disease is quite different, as it is a rapidly fatal disease of infancy, and in general hypercholesterolaemia, when it occurs, is of moderate degree.

Yet there is one reported case of a child aged five-and-a-half (Chevrel et al., 1937) which should probably be regarded as Niemann-Pick's disease running an unusually chronic course. The liver and spleen were grossly enlarged, and both histologically and chemically the lipoidosis was of the Niemann-Pick type. The blood cholesterol was 250 mgm. per cent., and there was no cutaneous xanthomatosis.

There may therefore be some relation between this syndrome and idiopathic xanthomatosis, since phospholipins and cholesterol are concerned in both. On the other hand the preponderance of sphingomyelin in the tissues is characteristic of the Niemann-Pick syndrome, whereas in the xanthomatoses the main deposit in the tissues is cholesterol ester, and in the plasma sphingomyelin and glycerophosphatide are both increased.

In conclusion, it may be said that although little is known of the true nature of the metabolic disorders in idiopathic xanthomatosis, the unusual case here reported should probably be classified as idiopathic xanthomatosis of the hypercholesterolaemic type with involvement of the liver, spleen, and kidneys, and is especially remarkable in showing enormously raised plasma phospholipins as well as cholesterol.

Summary

A case of juvenile xanthomatosis is reported, with arrested growth, enlargement of the liver and spleen, renal disease with hypertension, and enormous increases in plasma phospholipins and cholesterol.

The abnormalities in plasma and tissue lipoids in various pathological syndromes, and the classification of xanthomatous diseases, are discussed, and the case compared with others reported in the literature.

Thanks are due to Dr. A. G. Ogilvie for permission to publish this case and for the opportunity to carry out full investigations.

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ADDITIONAL MANIFESTATION OF SULPHATHIAZOLE TOXICITY*

NON-CALCULOUS RENAL INSUFFICIENCY FOLLOWING THE USE OF SULPHATHIAZOLE

BY

EDWARD PRESS, M.D., Englishtown, New Jersey, and MILTON KENNERSTEIN, M.D., New York City

(From the Children's Medical Service and the Pathology Department of the Lincoln Hospital, Bronx, New York)

'The possibility of serious renal damage resulting from sulphapyridine therapy, apart from the formation of calculi, lacks confirmation at the present time.' This statement was made by Smith, Evelyn and Nolan in 1940. A review of the literature reveals no disagreement with the above statement except for a single report of the finding of severe parenchymal damage in the kidneys of animals by Antopol and Robinson (1940). In all the reported studies and cases, whether calculous or non-calculous, anatomical changes in the kidneys were striking enough to account easily for oliguria or anuria, for hyposthenuria, and for nitrogen retention. The association of functional insufficiency and anatomical damage was obvious and direct.

The two cases to be reported here were characterized by marked evidence of renal insufficiency, i.e. oliguria and nitrogen retention, and evidence of renal irritation—the presence of albumin, casts, red blood cells, and white blood cells in the urine. However, on post-mortem examination, a striking absence of calculi and of anatomical findings in any way sufficient to account for the renal failure was noted.

Case Reports

Case 1. A. M., a three-and-a-half-months old Puerto Rican male, was admitted on November 19, 1940, because of fever, cough and nasal discharge of one week's duration. Birth had been normal and the subsequent history negative until the onset of the present illness. Physical examination revealed a moderately ill infant with a temperature of 100° F., pulse of 120, and respirations of 40 per minute. A profuse purulent nasal discharge, a markedly injected pharynx and hypertrophied and cryptic tonsils were present. Many medium and coarse musical râles were present throughout both lungs. The tympanic membranes were dull, but the landmarks were present. The urinary meatus was 2 to 3 mm. in diameter and allowed an uninterrupted flow of urine without apparent straining. The admission urine was completely negative with the exception of an occasional white blood cell. The blood count was as follows: haemoglobin 11·5 gm., red blood cells 3,570,000, white blood cells 12,800 with 64 per cent. lymphocytes, 4 per cent. eosinophils, and 32 per cent. neutrophils. The Wassermann reaction was negative.

During the first three weeks in the hospital the course was uneventful. Persistent nasal discharge and moderate injection gave evidence of the continued mild upper respiratory infection. The temperature, for the most part, was normal and rose on several occasions for a day or less to 101° F.

On the nineteenth day in hospital there was an elevation of temperature to 104° F., an increase in upper respiratory symptoms, occasional vomiting, and the appearance of three to five yellow-green stools daily. An examination at this time revealed a bilateral purulent otitis media. After twenty-four hours a bilateral myringotomy was performed which resulted in a purulent discharge from both ears. The culture from the left ear revealed a gamma streptococcus and from the right ear a staphylococcus aureus. The stool culture was negative and the diarrhoea ceased after the myringotomy. No evidence of pneumonia was found and the blood culture was negative, but the temperature persisted at 103–104° F. Therefore, on the third day of this elevated temperature, sulphathiazole was started. An initial dose of 1·2 gm. was followed by 0·3 gm. every four hours, producing a level of 4·1 mgm. per cent. After two-and-a-half days the temperature became normal, but at this time granular casts, red blood cells, white blood cells, and albumin were noted in the urine. The non-protein nitrogen was 75 mgm. per cent. and the blood pressure rose to 126/82 mm. Hg. Fluids were forced orally and parenterally and on the following day the non-protein nitrogen dropped slightly (to 65 mgm.) although the urinary findings persisted and there seemed to be some clinical improvement. However, in twenty-four hours the non-protein nitrogen rose again and the patient expired with a non-protein nitrogen of 123 mgm., five days after the onset of abnormal urinary findings and seven-and-a-half days after the beginning of chemotherapy.

AUTOPSY FINDINGS. The kidneys were of normal size for the age. The capsule stripped readily leaving a smooth surface with a few areas of congestion. Section showed cloudy swelling and congestion but no evidence of obstruction and no haemorrhage. No calculi or crystals were present. The renal pelves, the ureters and the bladder were entirely normal.

Other significant gross findings were as follows:

* Presented in summary at the Pathologico-Pediatric Conference in January, 1941.

The liver showed cloudy swellings and mild fatty changes. The small intestinal mucosa was mildly hyperaemic and oedematous. In both middle ears was about 1 c.c. of mucopurulent exudate and the right mastoid bone was necrotic. The meninges and sigmoid sinus were negative.

Microscopically the renal glomeruli were entirely normal, containing normal quantities of blood, no leucocytes or increased number of nuclei. The tubules showed pronounced epithelial degeneration with granulation and hyaline droplet degeneration of many cells, but no necrosis. Many tubules contained foamy or granular, very slightly basophilic material which was not calcium. The endothelium of many small vessels was swollen and a few contained small clusters of white blood cells. No thrombi were present. Frozen sections of unfixed tissue showed no evidence of crystal formation on direct and polariscopic examination. There were no other microscopic findings of note except that the mucosal stroma of the ileum was considerably thickened by an infiltration of lymphocytes, plasma cells, and a few neutrophils. The glands were compressed and scarcely secreting. Some of them contained some nuclear detritus.

Case 2. W. S., a five-months-old male infant, was admitted on November 26, 1940, because of diarrhoea, vomiting, and fever of one day's duration. The birth and development had been normal. One day before admission there was sudden onset of fever, the infant vomited three or four times, and passed eleven or twelve watery green stools without blood in twenty-four hours. There was no previous upper respiratory infection or any change in the formula. The physical examination revealed an acutely ill child with a temperature of 101° F., a pulse of 160 and Kussmaul respirations of 40 per minute. The fontanelle was sunken, the skin turgor only fair, and the child appeared moderately dehydrated. The physical examination was otherwise negative; there was no evidence of an upper respiratory or ear infection.

The urine on admission was completely normal, with no albumin and a negative microscopic examination. Blood count showed evidence of haemoconcentration with 16 gm. of haemoglobin; 7,200,000 red blood cells; 16,000 white blood cells, and 32 per cent. polymorphonuclears. The CO_2-combining power was 20 volumes per cent. Two successive stool cultures were negative for typhoid, salmonella, and dysentery bacteria. The Mantoux test was negative.

Parenteral fluids were administered and later a protein milk formula was given. After three or four days, paregoric and bismuth subcarbonate were added. The diarrhoea decreased to five or seven stools daily and the CO_2-combining power rose. Hydration improved greatly and the general condition was fair. On the third hospital day an upper respiratory infection developed and on the fifth day early signs of pneumonia were detected. These signs were corroborated by x-ray, and sulphathiazole was therefore started. 'Gag' culture revealed only streptococci and the blood culture was negative. An initial dose of 1 gm. of sulphathiazole, followed by 0·25 gm. every six hours, was given. After thirty-six hours during which a total of 2·25 gm. was given and the temperature dropped to normal, the sulphathiazole was discontinued because the urine showed a trace of albumin, occasional granular casts, 3 to 10 red cells and 5 to 12 white blood cells per high power field. At this time a scleremalous condition of the skin of the entire trunk and extremities developed, which in from one to two days progressed to a firm induration. The non-protein nitrogen was 151 mgm. Total proteins were 5·2 gm. Although serial x-ray films showed partial clearing of the pulmonary consolidation, the infant's condition gradually grew worse, oliguria developed and, in spite of a continuous intravenous infusion of glucose in distilled water and a blood transfusion, the non-protein nitrogen rose to 164 mgm. and the creatinine to 7·0 mgm. per cent. The patient expired five days after the onset of the urinary symptoms, six-and-a-half days after sulphathiazole had been started, and on the eleventh day in hospital.

AUTOPSY FINDINGS. Autopsy revealed kidneys that were moderately swollen. The capsule stripped easily leaving a smooth surface. Section revealed the appearance of cloudy swelling and in addition several small (1 mm.) haemorrhagic spots in the medullary portion of the collecting tubules. No petechiae were observed in the cortex. The pelves, ureters, and bladder showed a pale, smooth mucosa. They were of normal volume. No calculi or crystals were noted. The skin and subcutaneous tissue, especially over the lower abdomen and thighs, had a remarkably thickened appearance varying from 1½ to 2½ cm. in thickness. The subcutaneous fat was white, hard, and had the feel of beef suet. The lungs showed several grayish patches of consolidation most marked in the right upper lobe. The stomach and small intestine showed discolouration interpreted as autolytic.

Microscopic examination of the kidneys showed glomeruli well filled with blood, in some cases actually distended with blood. No leucocytes were present in the tufts or increased nuclei. The capsular spaces were of normal size and contained no exudate. The tubular epithelium was swollen and granular but not necrotic. Occasional tubules contained eosinophilic casts and granular debris. There were several large and some small interstitial haemorrhages in the medulla, pushing the tubules and blood vessels aside. Several thin-walled veins and their branches in the medulla contained completely or partially occlusive thrombi adherent to their wall. These consisted of heavy threads of fibrin between which were granular detritus, and masses of red blood cells, some showing haemolysis. With scarlet-R stain many tubules, both convoluted and collecting, contained collections of tiny fat droplets slightly in excess of normal. Unfortunately frozen sections were not performed unitl the tissue had been in 10 per cent. formalin solution for twelve hours, and although no microscopic crystals were found, there is a possibility of their dissolution. The sections of the skin showed a normal epidermis, the dermis appearing thickened by dense collagen. Although hair follicles were present, no sebaceous glands were seen. Sweat glands lay within the corium and appeared compressed. The subcutaneous fat layer was unusually wide and uniform. No inflammatory changes were present.

Discussion

Two cases are described: one, that of a three-and-a-half months and the other that of a five-

months old child, who upon treatment with sulphathiazole for an infection developed evidence of renal irritation, oliguria, and azotaemia. Since the infectious processes were limited and definitely not accountable for the deaths by themselves, it seems likely that the chief factor in the fatal outcome was the suppression of renal function. Although renal toxic degenerative changes were present at autopsy in both cases, they were not more severe than in the average infectious disease not attended by renal insufficiency (Kannerstein, 1942). No calculi or even crystals of acetyl-sulphathiazole were found in either case. In the second case, the presence of a few small haemorrhages and the failure to examine frozen sections of the kidney microscopically for crystals until after immersion in an aqueous solution may lead to questioning of the absence of crystals. However, the mere presence of a few microscopic crystals is certainly not a valid reason for renal insufficiency, especially when there was no evidence of obstruction. In short, the anatomical findings were entirely inadequate to explain the uraemia. It may be argued that the uraemia in these cases was pre- or extra-renal. There is no way of indisputably proving that this was not so; however, it seems very unlikely. In the second case, although initial dehydration was present, it was overcome long before the development of azotaemia, which increased progressively in spite of parenteral administration of large amounts of fluid. In the first case, dehydration was never a factor.

As mentioned above there have been practically no reports on renal insufficiency in humans as a result of sulphathiazole, in the absence of calculous obstruction. Antopol and Robinson (1940) from their work on animals suggested the 'possibility . . . that the drug may first produce parenchymal damage and subsequent to this, the acetylated compound is precipitated from the solution.' They also found that even when a dose insufficient to cause calculus formation was given, the kidneys still revealed the same degenerative glomerular and tubular changes. These changes were severe and included the presence of eosinophilic globoid bodies in the tubules, a coagulum in the loops of Henle and, in some cases, cuboid anuclear cytoplasm covering Bowman's capsule. One of the cases reported by Antopol (1940) reveals anatomic renal lesions in the absence of calculi or crystals in a middle-aged man who died of bronchopneumonia after two days of sulphapyridine therapy. Rake, Van Dyke, and Corwin (1940) noted that in the case of rats on sulphapyridine and mice on sulphathiazole there appears to be a primary damage to glomeruli and cells of convoluted tubules distinct from the changes due to the mechanical effects of the crystals. These changes were chiefly tubular necrosis and degeneration, collapse and destruction of tubules, and glomerular scarring. Although the above investigators suggest the possibility of anatomical renal damage due to sulphathiazole in the absence of obstruction, no previous reports of impairment of renal function without anatomical changes as a result of this drug have been found.* These cases are, therefore, reported as probable examples of the latter effect, in the hope that this will stimulate additional evidence for or against it, as well as an increase in caution in the use of the drug.

Summary

Two cases are presented which, during treatment with sulphathiazole, developed evidence of renal irritation, oliguria, and azotaemia from which they died. These disturbances were not accompanied by the formation of calculi or anatomic changes sufficient to account for them. It is suggested that this is a toxic manifestation of sulphathiazole causing a change in function rather than structure of the nephron.

Thanks are due to Dr. Harry S. Altman, attending Paediatrician to the Children's Medical Service, and Dr. Chester Brown, Director of the Pathology Department, for permission to report these cases and for helpful suggestions in their presentation.

* Since compiling this report two similar cases in adults were presented at conferences of the Mt. Sinai Hospital of New York City in February and April, 1941.

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BRITISH PAEDIATRIC ASSOCIATION

PROCEEDINGS OF AN EXTRAORDINARY GENERAL MEETING

An Extraordinary General Meeting was held at the Royal Society of Medicine, 1, Wimpole Street, W.1, on Saturday, December 12, 1942.

Business proceedings: The President, Professor L. G. Parsons (Birmingham), was in the Chair, and there were 40 members present.

Apologies for absence were received from Drs. J. Murray Bligh, R. W. Brookfield, Neil Hobhouse, F. John Poynton, A. G. Ogilvie, Eric Pritchard, James Rogers, Hugh Thursfield, E. R. C. Walker (now a prisoner of war) and K. D. Wilkinson.

The Minutes of the last Meeting held on May 29, 1942, were read and confirmed and signed by the President.

The following were unanimously elected Honorary Members:—

Dr. Charles Richard Box, Dr. Edward Alfred Cockayne, Professor Charles McNeil and Dr. Reginald Henry Miller,

and in addition Dr. R. R. Struthers of Montreal was elected a Corresponding Member.

After prolonged discussion, the question of the election of ordinary members and of admitting women to the membership of the Association was referred for further consideration at the next General Meeting.

The Secretary reported that the booklet was constantly being used as a means of introducing the Association to other bodies, and in view of this fact, it was suggested that the booklet when reprinted might have inserted against each member's name, his degrees and one appointment. It was resolved that this be carried out.

Treasurer's report. It was reported that in view of increased expenditure, £100 only had been invested in War Loan.

Secretary's report on general activities of the executive committee: This was received with much interest and it was resolved that it be circulated to all members.

You have had circulated to you a copy of the report on the discussion which took place at Windermere on May 29, 1942. At that meeting it was proposed by Professor J. C. Spence and seconded by Dr. A. Ogilvie 'That this Meeting instruct the Executive Committee to interest themselves in and advise any appropriate authority in current Paediatric Problems, and for this purpose be empowered to co-opt members both from the Association and other bodies.' Current problems for the Executive Committee to consider and take action upon were :—

(a) Nutrition (with special reference to vitamin 'D'), Neonatal Mortality, Day nurseries, Breast feeding in relation to female labour, Juvenile delinquency, Safe milk and the Early diagnosis of tuberculosis.

You have received a copy of a memorandum on all these subjects, and it is these which will form the basis of our discussion during the morning session. The Ministry of Health, the Ministry of Food and the Board of Education acknowledged the reports and we are meeting Representatives of the Ministry of Food in the course of the next month with regard to the vitamin 'D' report.

You will remember it was also agreed that the possibility of a survey of the incidents of rickets throughout the country be discussed, and your Executive have appointed Drs. A. Moncrieff and S. Graham to organize such a survey. The cost of this survey is being met by a special grant from the Ministry of Health, and it is taking place in twenty different centres, during the six weeks from the middle of January to the end of February, 1943. X-rays of the wrists of infants between three and eighteen months are to be taken and a detailed questionnaire completed for each child. Sufficient copies of this questionnaire will be sent to each centre. The x-rays will be examined by a committee of radiologists and the facts obtained in the questionnaire analysed by Dr. Percy Stocks. It is hoped to obtain up to two hundred children at each of the twenty centres. Dr. Percy Stocks is statistician to the Ministry of Health.

(b) Future paediatric policy, with special reference to the memorandum prepared by the London members of the association: You will have received a copy of a report submitted by Professor J. C. Spence to the Nuffield Trust, and also a report prepared by Professor L. G. Parsons on 'Suggestions for the improvement of Teaching in Paediatrics in the University, and for the Provision of a Paediatric Service for Birmingham and District.' You also received a copy of the Memorandum submitted by the Association to the Interdepartmental Committee on Medical Schools.

A Deputation, composed of Professors Parsons and Spence and Drs. Capon, Moncrieff, Paterson and Wyllie, waited on the Interdepartmental Committee on Medical Schools, on Wednesday, October 28, 1942. They gave evidence in support of the Memorandum, and it may be stated that they were thanked and complimented on the well-organized way in which their evidence was presented. Professor Charles McNeil gave evidence before the Interdepartmental Committee when it visited Edinburgh, on much the same lines as the evidence given by the Executive Committee in London. The Association was asked by the Interdepartmental Committee to prepare a Memorandum on 'Accommodation for Newly-born Infants in Maternity Hospitals,' and this report is in process of preparation and discussion.

(c) Paediatric aspects in 'Group Practice' or similar developments arising out of the forthcoming report of the B.M.A.'s Planning Commission: You will have received the Memorandum prepared by the Executive, on the Draft Interim Report of the Medical Planning Commission of the B.M.A. Professor Charles McNeil and Dr. F. M. B. Allen are carefully watching the interests of paediatrics as members of the Planning Commission.

(d) The sending of a Resolution to the General Nursing Council, the College of Nursing and the Nursing Division of the Ministry of Health, strongly urging the retention of the special register of Sick Children's Nurses: This whole matter is under consideration, and much correspondence has passed back and forth. The Executive Committee have continued to protest against any attempt to do away with this Register.

(e) What part the British Paediatric Association should take in child relief in Europe: You have received a circular letter on this subject, and the sub-committee dealing with this matter are awaiting your reply before taking further steps. In future, this is to be known as the Sub-Committee for Continental Child Relief.

(f) That Paediatrics should form a more important part in the final examination of Students: In August of this year, all the Universities and Examining Bodies were written to, deploring the lack of interest in and prominence given to diseases of children in the final examination of the medical curriculum, and urging that, in view of the growing recognition of the importance of this subject in the health of the nation, much more attention be

paid to this aspect of medicine by those responsible for conducting the examination. The President undertook to raise this question at the General Medical Council. A number of replies were received which were most unsatisfactory. The three most satisfactory replies up to date came from Glasgow, Belfast and Liverpool, and it was suggested to various of the examining bodies that they might like to adopt some of the measures taken by these three Universities. The Royal College of Physicians has asked for a Delegation from the Association to meet their Planning Committee and discuss this question during the month of January, 1943.

Conference: The organization of the Conference which is to take place this afternoon with members of the following : the Public Health Services, Medical Officers of Schools Association, Children's Section of the Royal Society of Medicine and Representatives of the B.M.A. has occupied much of your Executive's time and thought. Various important officials of Public Bodies mentioned on the Agenda have been invited. The Draft Agenda was revised after a Conference with representatives of the Society of Medical Officers of Health.

Other Activities:

Members will have received a copy of the Association's Booklet, 1942/43. About one hundred copies of this booklet have been circulated with various letters and reports introducing the B.P.A.

The President contributed a most valuable article to *The Times*, under the title of 'The Children's Health,' on September 12, 1942, which many of you will have read with pleasure.

Members will also have noted with satisfaction that publicity has been given to various of our Memoranda in the Medical Press.

The Executive Committee has been in touch with the Child Guidance Council, and the Association's policy is that the setting up of Child Guidance Clinics should be done in close association with the Children's Departments of Teaching Hospitals or in Children's Hospitals themselves, and that such clinics should be staffed as recommended by the Child Guidance Council, i.e. with fully-trained psychiatrists, psychologists and social workers with psychiatric training, working in close co-operation with the paediatrician.

Rheumatism: The Executive is contemplating a Memorandum on Rheumatism, and various members of the Association will be asked to take part. The Secretary of this Sub-Committee is Professor Bruce Perry.

Your Executive Committee met in July, September, October, November and December. The total time during which they were in session at their meetings exceeded twenty-five hours, and the various members travelled a total distance of more than 11,000 miles. Since we last met on May 29, 1942, over 800 letters have been dispatched, either containing reports or in answer to communications, and the number of words in the memoranda which each member has received have exceeded 25,600.

Honours: Our President was congratulated by the executive committee on your behalf on being awarded by the Royal College of Physicians the Moxon medal, which is awarded every third year to the person who is deemed to have most distinguished himself by observation and research in clinical medicine, and also on being appointed first Charles West Lecturer—a lecture on Diseases of Children to be given every third year.

The Executive, on your behalf, also congratulated Professor J. C. Spence on his appointment as Professor of Child Health at Durham University.

Letters of thanks from the executive committee have been sent both to members of the Association and co-opted individuals who have assisted so whole-heartedly in the preparation of the various memoranda and reports.

My grateful thanks are due to Dr. Alan Moncrieff for much help in my Secretarial work, and to my Secretary, Miss N. Hair, for her untiring efforts on the Association's behalf.

(Signed) DONALD PATERSON.

12th December, 1942.

It was resolved that the date and place of the next meeting be left to the Executive Committee.

Scientific proceedings: A report on the Early Diagnosis of Tuberculosis in Childhood was received and discussed. It was referred back to the sub-committee for reconsideration. Other reports, previously circulated, were discussed. These included reports on Breast feeding in relation to female labour, Safe milk, Juvenile delinquencies, Neonatal Mortality, The dosage of vitamin D, War-time Nurseries. (Some of these are printed below).

REPORTS OF SUB-COMMITTEES

At the Fourteenth Annual Meeting of the British Paediatric Association it was decided to instruct the executive committee to investigate certain problems. This has been carried out by various sub-committees and individuals, and the reports in each instance have been passed by the executive committee and submitted to the general meeting held in London on December 12, 1942.

NEONATAL MORTALITY

BY C. MCNEIL (EDINBURGH—CHAIRMAN), N. B. CAPON (LIVERPOOL—SECRETARY), W. R. F. COLLIS (DUBLIN), S. GRAHAM (GLASGOW) and A. MONCRIEFF (LONDON)

1. We wish to make it clear, at the outset, that when we speak of neonatal mortality we have also in mind (a) the no less important, though less sharply defined question of neonatal morbidity; and (b) the loss of potential lives by stillbirth. In regard to neonatal morbidity, it should be stressed that though young infants possess a remarkable power of recovery from trauma and illness, failure to make **full** recovery is especially regrettable in early life, when each individual should be entitled to the prospect of a long term of good health.

2. It is common knowledge that the gratifying decline in the infant mortality rate which has taken place in most countries during the past fifty years or more has not been accompanied by a correspondingly satisfactory fall of the neonatal mortality rate. Yet some countries and areas (e.g. Holland, Australasia, and the large cities of the U.S.A.) have been more successful in this respect than others (e.g. Scotland, England, and Wales), which shows that the problem is not insoluble.

3. In studying neonatal mortality, it is important to view the particular epoch in its relationship both to preceding events and to subsequent life. Without entering into detail, it is apparent that adverse influences may operate:

(a) Hereditarily.
(b) During embryonic life, e.g. congenital malformations.
(c) During foetal life, e.g. syphilis.

(d) By premature expulsion of the foetus in an immature state.
(e) During birth, e.g. birth-trauma; and failure of the physiological readjustments consequent upon birth.
(f) After birth, during the neonatal period, e.g. infections, lack of adequate nursing skill and care, and mismanagement and failure of breast-feeding.

4. Thus it is obvious that the problems of neonatal mortality concern not only paediatricians, but also obstetricians, general practitioners, general physicians and public health workers, and there is a need for close co-operation in study and practice. But the onus of leadership of the team should be accepted by the paediatricians who are more intimately concerned than any other group.

5. In this report we shall restrict our observations to the chief causes of neonatal mortality, which are:
(a) Immaturity (usually prematurity).
(b) Birth-trauma (especially intra-cranial haemorrhage).
(c) Respiratory difficulties arising during or immediately after birth.
(d) Infective and toxic conditions.
(e) Congenital malformations.

6. (a) To give some idea of the neonatal mortality rates per 1000 live births in different countries, the following figures are quoted, and the year to which the figure refers is given in brackets.

(By neonatal mortality is meant deaths during the first month of life.)

Adelaide	(1936)	20
Holland	(1938)	21
Greater London	(1925)	26
,, ,,	(1937)	24
England	(1938)	26
Chicago	(1925)	37
,,	(1937)	25
Scotland	(1938)	35
Glasgow	(1937)	43

(b) In order to give some idea of the total numbers of deaths involved, the following facts may be of interest:

Total number of neonatal deaths in England and Wales each year about	18,000
Total number of stillbirths in England and Wales each year about	25,000
Total number of neonatal deaths in U.S.A. each year about	70,000
Total number of stillbirths in U.S.A. each year about	72,000

7. **Immaturity**. This accounts for about 50 per cent. of neonatal deaths and about 50 per cent. of stillbirths. In many cases it is the primary cause of death, in others it constitutes a strong predisposing cause, because immature babies do not tolerate birth-trauma and infections as well as mature babies; their respiratory transition is hazardous and they are particularly susceptible to lack of specialized nursing care. Those who survive are prone to develop anaemia, rickets and malnutrition, especially in the less-favoured classes.

The prevention of immaturity is a problem of major importance and we recommend that new efforts should be made to solve it.

The treatment of immaturity requires special paediatric care, supported by skilled nursing (specially trained staff) in suitably equipped units; there is evidence that the usual death rate of about 50 per cent. can be reduced in this way to less than 20 per cent. The chief aims of treatment are:

(i) Maintenance of steady temperature and prevention of dehydration by special heating appliances or conditioned air.
(ii) Correct feeding (high calorie feeding and liberal fluid allowance), gavage often being necessary.
(iii) Skilled nursing by a specially trained staff.
(iv) Isolation from all possible sources of infection by provision of special isolation nurseries.

A premature station or unit should be set up in every maternity hospital, and in all important children's hospitals; they should be in the charge of paediatricians possessing experience of neonatal work. Their value would not be limited to the care of the patients; they would be important teaching centres for undergraduates, postgraduates, midwives, children's nurses and health visitors.

8. **Birth trauma**. It is obvious that attention should be centred mainly upon prevention, which is a problem for obstetricians, general practitioners, doctors in charge of ante-natal clinics, and midwives in private practice. Paediatricians can assist by supporting all measures designed to secure first-class obstetrical practice.

9. **Respiratory difficulties**. The incidence of asphyxia neonatorum could probably be reduced by:

(i) Securing a higher standard of ante-natal care.
(ii) Consultations with obstetricians and physiologists in order to reach conclusions upon such points as:
(a) The use of analgesics and sedatives; and
(b) Details of ' first-aid ' care of the infant including methods of resuscitation.

We desire to call attention to the lax use of the term ' asphyxia neonatorum ' on death certificates, and there is little doubt that many examples of birth-trauma, neonatal pneumonia, and other conditions giving rise to (secondary) asphyxia masquerade under the title. A solution is difficult to see, but we recommend that the attention of the Registrar-General be called to the point. We also urge the importance of post-mortem examinations in cases of this type.

10. **Infective and toxic conditions**. Both the medical and nursing professions are still sadly ignorant about the prevalence and danger of neonatal infections, and there can be no efficient prophylaxis until this shortcoming is removed. The dangers are particularly great in institutions. Neonates should not be herded together, and this is especially necessary for those newly-born babies which are particularly prone to infections, e.g. prematures, weakly infants and those suffering with birth-trauma or congenital malformations. These should be nursed under the direction of an expert paediatrician in a special unit preferably designed on the cubicle system, and should be tended by a specially trained nursing staff, employing modern methods of prophylaxis including gowns and masks. There should be a wash-hand basin in each cubicle. Nurses responsible for changing the babies should not prepare nor give the feeds. A modern sterilizing apparatus should be used to disinfect napkins. A milk-kitchen is required, not only to ensure that artificial feeds are prepared accurately, and bottles are thoroughly sterilized, but to enable the nurses to receive adequate tuition in this important branch of the work, which should be supervised by a trained dietitian working under the control of the paediatrician. Breast milk is especially necessary for babies suffering with infective conditions (see para. 12), and the diets of the mothers require particular supervision (see para. 13).

11. **Congenital malformations**. In the present state of knowledge it is not possible to prevent congenital malformations; further research is necessary, particularly in regard to the possible influence of therapeutic agents, e.g. x-rays and radium.

12. **Breast-feeding**. An adequate and continuing supply of breast milk should be available for every infant, and particularly for immature, weakly, birth-damaged and ill babies. It is a tragedy that many infants are given artificial feeds, on the advice of doctors and nurses, without sufficient justification. This fact provides a strong case for better and more vigorous instruction.

Not infrequently the supply of breast milk fails when the mother enters upon her home duties again. Planning and supervision by the family doctor or home visitor or post-natal clinic would frequently prevent this failure, particularly if the mother's diet were to receive careful attention (see para. 13), and if she were able to take sufficient rest. Too speedy a return to strenuous household duties such as washing, cooking and cleaning, results in tiredness, worry and failing health (especially anaemia); it is not surprising that breast-feeding often fails under these conditions. The psychological aspects of breast-feeding, which frequently breaks down because the mother is over-anxious, lacking in confidence, or worried, require careful consideration and should be stressed in the course of instruction given to medical students and nurses.

Breast milk banks or pools, set up in maternity hospitals or other suitable centres, would be of great value to the surrounding district and should not be difficult to maintain: the blood banks started in recent years under the stimulus of war conditions have shown what can be done by concerted and vigorous effort.

13. **Diets for mothers**. The health of a newly-born baby is greatly influenced by the state of the mother's health during the ante-natal period. Diet, hygiene, attention to minor ailments (e.g. dental caries and secondary anaemia) and freedom from worry are some of the subjects which need consideration by the family doctor or the ante-natal clinics. We shall speak only of diet, to which much attention has been directed of late. There is evidence that satisfactory dieting during pregnancy diminishes the incidence of still-births, promotes the general well-being of neonates and improves the mother's capacity to provide breast milk. For the poorer classes it should be possible to ensure that every pregnant woman receives a well-balanced diet of some 2500 calories with sufficient vitamins and salts each day for the three months before confinement and throughout the period of lactation. In most districts these meals could be taken in the home, but in the poorer ones it might be more suitable for one meal of roughly 900 calories to be provided each day at a communal feeding centre.

14. **Some general recommendations**. (a) A committee composed of paediatricians, obstetricians and public health experts (with power to co-opt other specialists as necessary) should be set up to discuss all aspects of neonatal mortality and morbidity, and to direct the further investigations which are necessary. The most important of their tasks would be to consider:

(i) The health of the pregnant woman.
(ii) The relative advantages of domiciliary and institutional confinement.
(iii) Breast-feeding.
(iv) Prevention of immaturity.
(v) Prevention of maternal and neonatal infections.
(vi) Birth injuries.
(vii) Respiratory difficulties at birth.

This committee should be empowered to advise the Minister of Health, in order that effective action may be taken.

(b) Undergraduates, postgraduates, midwives, children's nurses and health visitors are all in need of more tuition upon neonatal health and disease.

The Central Midwives Board should include at least one paediatrician, and the final examination should include questions on neonatal conditions set by a paediatrician, as is the custom in Northern Ireland.

(c) Each maternity hospital and most of the larger children's hospitals should have a properly equipped and efficiently staffed neonatal unit. These units would form part of the Department of Paediatrics in the region, and junior paediatricians would thus have better opportunities for clinical experience of the particular age-period. The sisters and senior nurses working in neonatal units should be qualified both as midwives and children's nurses, and their salaries should be raised accordingly.

(d) It is hoped that the setting up of a committee as recommended above would ensure the close co-operation between the Ministry of Health and local health authorities on the one hand, and paediatricians on the other, which is essential for the efficient handling of the problem.

(e) Uniform standards for statistical returns from maternity hospitals and maternity nursing homes should be secured, and there should be a more general adoption of exact standards of immaturity or prematurity, and careful and accurate returns of still-births.

THE STANDARD OF MILK SUPPLIED TO CHILDREN

By A. G. WATKINS (Cardiff)

(This report was based upon evidence obtained from eight areas, urban and rural. Because of the confidential nature of the replies received to a questionnaire it is not possible to publish the figures obtained.)

1. No available evidence of increase in milk-borne diseases since the war. The shifting population makes accurate statistical figures difficult to obtain, though in some areas there is a clinical impression of an increase in tuberculous glands of neck.

2. Definite lowering of bacteriological quality of milk since the war.

3. Frequent comments by medical officers of health on the unsatisfactory pasteurization of milk.

4. Likely decrease in standard of milk, especially keeping qualities, due to war-time transport problems.

Recommendations:

1. All pooled milk (other than 'reject' and 'salvage' C.M.D. 6362) to be **efficiently** pasteurized. Increased inspection of cooling facilities on farms and of pasteurization technique.

2. Until all milk is efficiently pasteurized, the public should be instructed to scald all liquid milk for children up to at least five years of age, but preferably dried milk should be used for infants.

3. These investigations reveal that the safeguards to ensure that pasteurization is efficient are not reliable, and it is recommended that steps be taken to remedy this state of affairs.

WAR-TIME NURSERIES

By K. D. WILKINSON (Birmingham)

In the City of Birmingham there are at present (October, 1942) 52 nurseries in operation, accommodating 2753 children, and a further 29 are in process of formation for 1659 children. In the Midland Region (No. 9) there are:

In preparation	9	nurseries for	430 children.
Approved ..	82	,, ,,	3953 ,,
Operating ..	103	,, ,,	4946 ,,

Under present conditions the situation of the nurseries is governed by such considerations as suitable premises

and the neighbouring homes of large numbers of factory workers. The result is that the nurseries have to make shift with houses which from many points of view are not ideal, and premises which have been adapted to the purpose of the nursery without great structural alteration, by the addition of baths and lavatories, and modifications in the kitchen. Indoor and outdoor play space is often limited, especially outdoor under-cover space.

The houses, schools, and other buildings are attractively clean and many have pictures and decorations of good type.

Most of the nurseries take children each day from 7 a.m. to 7 p.m. A few take children for the whole week, since the mothers are on night shifts, the children going home on Saturday and returning on Monday morning.

The children are of all ages up to five years. Some nurseries have children up to one year. Most have toddlers one to two years and seniors two to five years.

The proportion of the various groups determines the number of staff required, since up to two years the children require a lot of personal attention, washing and feeding, and after that age do most things for themselves under supervision.

I have seen the best and the worst nurseries under varying conditions of weather, and at various times of day.

Most nurseries have separate playrooms for seniors and juniors, and also separate sleeping rooms in which each child has a stretcher and two blankets marked with a name and a diagram (a blue rabbit or a red lion, and so forth) by which they are easily identified. In the playrooms are a number of indoor toys—bricks, wooden devices of various kinds, simple jigsaw puzzles. There is not enough variety as a rule, but the number of toys varies considerably at different centres. The outdoor equipment for games—chutes, climbing woodwork, boats, carts, is often quite inadequate, but in some is generous.

Washing and lavatory accommodation is good.

Food. The small children are given milk feeds—almost always prepared from dried milk—and, in addition, get an emulsion containing vitamins A, C and D, so arranged that a teaspoonful contains 6000 A units, 1200 D units. Breast-feeding is rare—I only saw two children so fed in several hundred—but on the other hand it is uncommon to have children under three months of age in the nursery.

Older children get a varied midday meal in addition to food at about 11 a.m. and 4 p.m. These also get the vitamin emulsion in larger doses. The midday meal is obtained from varying sources. The kitchen at the Children's Hospital feeds the nursery there and two other nurseries. Food is cooked at a central kitchen in some instances and distributed in heated containers. Many of the nurses cook their own vegetables and obtain meat from a kitchen. In a few instances a British Restaurant supplies the food.

The nursery at the Children's Hospital is on the second floor—all on one floor. It has 80 children on the books—20 under one year, 20 between one and two years, and 40 over two years of age. The average daily attendance is 60. One of the babies is breast-fed three times a day at present. There are 24 women staff. This includes a matron, who is a trained nurse, several with Nursery Certificates, and others in training. Their ages vary from fifteen upwards. They appeared neat and keen on the work.

Nursery 'A' is housed in a school. It has been lavishly redecorated and is spacious. Situated in the worst slum area in the city, it is particularly well equipped in all respects, and is used as an observation and training centre for teachers, and for psychological instruction of nurses and teachers.

On the other hand, the Nursery 'B' is situated in a house which is essentially unsuitable, since individual rooms are small and there are three floors in use with steep stairs. This deals with twenty small children and has a staff of eleven. It was there that the difficulties presented by a shortage of elastic were obvious, for replacements of this material offer great difficulties, and clothing is largely suspended by elastic.

Nursery 'C' takes 53 children from two-and-a-half to five years, and has a staff of 9. Outdoor toys are very poor. Indoor toys were very good.

Nursery 'D' is a resident nursery which inhabits a school and has good accommodation. The children number 65, of whom 7 are under one year. None is breast-fed. The staff is 15 in number. There is a garden, but there is no provision of any kind of outdoor toys for these children.

It seems to me fair to say that the Day Nurseries, as at present constituted, fill a large gap in providing care and attention for children while their mothers work, that the food is good, the supervision adequate.

I have been very much struck by the kindliness and cheerfulness of those who are doing all sorts of jobs at these Nurseries; in the course of my travels I have not seen a child in tears or heard a cross word. The only complaint was by a boy, who, having had three helpings of fish pudding, said he did not like fish!

The accommodation in many instances leaves much to be desired. Infection does occur and whooping-cough, chicken-pox and measles have been sources of trouble at one time and another. The risks of exposure are not greater in the Nursery than they are in the child's own home, since the majority of these children come from homes in which supervision is very poor.

I feel that these Nurseries are of the greatest use to the parents and a real benefit to the children. The food which is distributed to the inmates is an addition to the rations which their parents obtain for the family at home.

Basis of diet

BEFORE DINNER: Each child to have one rusk (½ oz. of bread cut in strip and crisped in oven).

AFTER DINNER: Each child to have portion of carrot or apple to chew.

	Per day	*Per mid-day meal*	*Per mid-day meal with two bottles of milk*
Calories required per child	1500	700	—
Calories allowed per child	—	633	873
Gm. protein required per child	52	20–25	—
Gm. protein allowed per child	—	18·3	30·9
Gm. fat allowed per child	—	14·1	29
Gm. calcium allowed per child	—	824 mgm.	—
Gm. iron (available) per child	—	3·9 mgm.	
Allowance of meat	1d.	per head per meal	
,, potatoes	4 oz.	,, ,,	
,, root veg. or green veg.	2 oz.	,, ,,	
,, bread as rusk	½ oz.	,, ,,	
,, egg	½ egg	,, ,,	

Note. The Executive Committee of the British Paediatric Association considered this report on War-time Nurseries in the Birmingham area, and as a result of these considerations and verbal reports from other areas, makes the following recommendations:—

1. As far as possible, posts as Matron or Sister in these Nurseries should be filled by trained nurses who preferably have received a children's nursing training.

2. In view of the increased risk of infection to a child in a War-time Nursery, it is strongly urged that a condition of admission should be immunisation against diphtheria.

3. It is also strongly urged that only 'safe' milk should be used in War-time Nurseries; by 'safe' milk is meant milk which has been dried, efficiently pasteurized or boiled.

THE DOSAGE OF VITAMIN D

By W. SHELDON (London—Chairman), C. F. HARRIS (London), N. MORRIS (Glasgow), and HELEN MACKAY (London—Co-opted)

1. Opinions on vitamin D requirements of infants and children

(a) The minimum requirement for infants and children is not known.

(b) The Technical Commission on Nutrition of the League of Nations (1938) place the requirements of infants at 400–500 i.u. daily.

(c) The Committee of Food and Nutrition of the National Research Council in the U.S.A. and the Committee on Vitamins of the American Academy of Paediatrics (Dann and Davison, 1942) put the requirements of children from birth up to fifteen years of age at 400–800 i.u. daily.

(d) The figure in (c) has been endorsed by the National Nutrition Conference for Defence (1941) summoned by President Roosevelt.

(e) Eliot and Park (1942) consider that 800–1000 i.u. daily cover any possible requirement of full-term infants.

(f) At St. Bartholomew's Hospital, London, for the last ten years, it has been the practice to give daily doses of 600 i.u. as cod-liver oil to infants prophylactically.

(g) Premature infants (i.e. infants whose birth weight is below 5½ lb.), vary more widely in their individual requirements than full-term infants, but their need is uniformly higher. Their daily routine allowance should be twice that of full-term infants, and should be continued at this rate until their second birthday.

(h) The incidence of active rickets is as great under six months of age as over. In view of the more active growth rate in the earlier months of life, the prophylactic requirement of vitamin D is greater in these months.

(i) In summer in Great Britain, sunshine and skyshine can generally be relied upon to protect full-term infants from rickets, provided they are suitably exposed to those elements for at least four hours daily. This provision cannot, however, be expected to be uniformly available, especially under the difficult conditions of war-time. In winter little vitamin D is rendered available by natural irradiation. This sub-committee therefore considers that seasonal variations in vitamin D allowance would be unwise.

(j) With regard to children beyond the age of infancy, who are receiving a mixed diet, the amount of vitamin D obtainable from their food is subject to such variation and, in any case, is so small that a deduction in the amount of vitamin D supplement is considered to be unjustified.

(k) With regard to infants, this report does not distinguish between those who are breast-fed and those artificially reared, the sub-committee being of the opinion that a supplementary ration of vitamin D should be issued impartially to both groups of infants, irrespective of whether or not nursing mothers are to be allowed a vitamin D supplement.

2. The present official dosage of 'cod-liver oil compound'

The cod-liver oil compound supplied by the Government as a supplementary ration for children under five years of age, contains ' not less than 100 i.u. of vitamin D per gramme.' The maximum dosage for children under six months old is ' half a teaspoonful daily,' and for those over six months, ' one teaspoonful.' On a generous interpretation, this provides 200 i.u. daily under six months of age, and 400 i.u. daily for older infants. It would be hard to find any authority with clinical experience to support so small a dose. Moreover, it will be noted that infants under six months are allowed a smaller dose than older babies, which does not accord with their actual needs.

3. Recommendations re dosage of vitamin D

A daily allowance under the Government scheme should be sufficient to protect practically all babies under the varying circumstances of their lives, without further addition of vitamin D, obtained through private channels.

(a) Full-term infants and children up to five years old.

Although a daily intake of 500 i.u. would probably protect the majority of full-term infants and young children, a daily allowance of 700 i.u. would give a wider margin of safety. For simplicity in administration, a ' vitaminized ' oil should be so reinforced with irradiated ergosterol that 1 fluid drachm contains the daily allowance of 700 i.u.—as well as the 3500 i.u. of vitamin A now provided in each drachm.

(b) Premature infants (under 5½ lb. at birth) from birth to two years.

The allowance should be double that in paragraph 3 (a), i.e. a daily allowance of 1400 i.u., contained in 2 fluid drachms of ' vitaminized ' oil.

(c) School children.

Information regarding the vitamin D needs of school children is meagre, although there is much to suggest that vitamin D deficiency is one factor in the etiology of dental caries in our school children. Taking also into consideration the small amount of fat, fish, eggs and meat fat that are available, it is recommended that a supplement of 3500 i.u. of vitamin D per week be allowed for this group of children. Administratively, this supplement might be issued with school milk, or through the medium of school dinners.

(d) Pregnant and nursing women.

It is recommended that a supplement of 700 i.u. of vitamin D daily be allowed to this group of the community. The provision of such a supplement should not do away with the supplement for breast-fed infants.

4. Suggestions concerning routine administration of vitamin D

Printed directions for the mother should be issued with the ration of vitamin D. These should include :

(a) Instructions to start the oil gradually, beginning with a few drops daily when the infant is a month old, and taking a fortnight to work up to the full dose of one teaspoonful (700 i.u. daily).

(b) Inform her that for an infant whose weight at birth is over 5½ lb. the supplement of vitamin D need not be given on summer days, provided the infant is out of doors morning and afternoon.

(c) Warn her that an infant whose birth weight is below 5½ lb. is particularly susceptible to rickets; that the allowance of vitamin D supplement must therefore be doubled to 2 teaspoonfuls daily, and that this should be divided into four equal doses each day, and begun gradually; and that this supplement should be given throughout summer and winter; and that all premature babies should be under regular medical supervision.

For the information of doctors, the composition and vitamin content of ' cod-liver oil compound ' should be given on the label, and published in the medical press.

5. Recommendations to the executive committee of the British Paediatric Association as to procedure that might be adopted with this report

(a) Assuming that the executive committee accept this report, it should be forwarded to the Minister of

Health, the Minister of Food, and the Minister of Education, asking for their co-operation in putting the recommendations into practice.

(b) That either the whole report, or an abstract of the recommendations, be published in the medical press, including the public health journals, and be sent to the nursing journals.

(c) That copies be circulated to the members of the British Paediatric Association, and to the members of the Society of Medical Officers of Health.

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BREAST-FEEDING IN RELATION TO FEMALE LABOUR
AS IT PARTICULARLY AFFECTS BIRMINGHAM

By A. V. NEALE (Birmingham), ETHEL CASSIE (Birmingham, co-opted), FRANCES BRAID (Birmingham, co-opted) and MILA PIERCE (Chicago, co-opted)

Following a request from the executive committee of the British Paediatric Association, in July, 1942, a committee was formed, to investigate breast-feeding, in the City of Birmingham, with a special reference to the relationship to female labour. We are greatly indebted to the Health Visitors (100) working in the Department of Maternity and Child Welfare. Those visitors were asked to report on the babies born during the first quarter of 1941, and of 1942. Each Health Visitor was given a form setting out the information required, as detailed in the questionnaire, as follows:

QUESTIONNAIRE TO COVER RETURN FORM

First or later child.
Period of breast-feeding.
Mother working or not during pregnancy.
If so how long.
Mother working after confinement.
If so how soon.
If not, is she anxious to commence, or does she wish to postpone until normal breast-feeding period is over.

They also received a personal explanation from the Superintendent Health Visitor, in regard to the exact requirements, special emphasis being given to the reasons why the information was wanted, in order to obtain enthusiastic co-operation. The schedules were to be returned within six weeks. At the end of the period, those schedules which had not been sent in were looked up, and a certain number had to be returned for further information. The Superintendent of the Health Visitors incorporated the schedules to obtain the figures utilized in this report. We emphasize that this required considerable extra efforts in the Health Visitors' work. Records of 4635 babies were obtained. In addition to the statistics some comments are included, which were obtained in a personal discussion, by Dr. Ethel Cassie at a general meeting of Health Visitors; the observations set out were unanimous.

Notes on Discussion with Superintendent Health Visitors, 19th February, 1942

1. The incidence of breast-feeding is not materially affected by the present situation. In the areas where married women were not accustomed to working in the factories, they are not going into the factories while they have a baby under one year. In the areas where they were accustomed to working before the war, the women go out as before, but usually at no earlier date, i.e. they wait until the baby is a couple of months old at least. This is borne out by the admissions to war-time nurseries. Young babies are coming in very small numbers. It is only in poor districts that the number of babies is more than one-fifth of the total. Even then, it is rare to have an infant under three months.

2. Present influences, which are adverse to breast feeding are:

(*a*) Queueing for shopping.

(*b*) Emotional disturbances, particularly anxiety as to husbands.

(*c*) Part-time work, liability to supplementary feeding.

(*d*) Doctors are still responsible for weaning babies more than any other single factor. They still take babies off the breast, whenever there is any difficulty, rather than take the trouble to go into the whole difficult question of successful breast-feeding. Doctors were named who made a practice of this.

Babies are doing very well, better than before the war, and the general feeling is that breast-feeding has not gone down.

Where the men are at home, and on munition work, as they are in large districts of the City, there is more money going into the homes, and the women are in such circumstances disinclined to go out to work, and the infants are, on the whole, better cared for than in the past.

A further enquiry was made by Dr. Mila Pierce in the out-patients' department of the Birmingham Children's Hospital, and her findings are included in the report.

We should state that a prior enquiry was made in 1938, in reference to babies born in January to June, 1937. A copy of these statistics are enclosed in this report (table 1).

It is interesting to note that the incidence of breast-feeding (wholly or partly) for three to six months, was 51 per cent., whereas in the 1941 and 1942 enquiry, it was practically the same—53 per cent. This indicates that in the pre-war periods there appears no obvious factor altering the incidence of breast-feeding to any appreciable degree. Some observations were made in 1938 in regard to the reason for the limited incidence of breast-feeding or its abandonment. Table 2 indicates the details.

It will be noted that in the 1939 series, 15 per cent. gave up breast-feeding because of going to work. In the present series, only 11 per cent. gave a similar reason. This would seem to be a reflection of the diminished family financial embarrassments in the munition worker group.

Comparison shows that in the earlier weeks the first baby is at a disadvantage as compared with the second or later baby; but after two months this difference disappears. This may bear some relationship to a lesser degree of maternal anxiety in the second or later pregnancy.

Working in pregnancy appears to have little influence in regard to subsequent breast-feeding. It is interesting to note that only a small proportion are anxious to return to work; and these are mainly unmarried mothers or women in key positions. The actual numbers returning to work are small, and only amount to approximately 12 per cent.

Since only 12 per cent. are working after confinement, it appears that women, as a whole, prefer to stay at home with their infants when economic circumstances are favourable. There is corroborative evidence of this in Dr. Pierce's cases. The same point is revealed in the small number of young infants admitted to Day Nurseries, although they are accepted from the age of one month. It has been noted that the only nurseries where the larger

TABLE 1

BREAST-FEEDING RECORD OF CHILDREN BORN JANUARY TO JUNE, 1937
(Children living January, 1938)

Total number of cases reported by Health Visitors	(1) Inner circle 12 centres 2023	(2) Outer circle 21 centres 2355	(3) Total of Col. (1) and (2) 4378	Total per cent. of all babies of corresponding age
Breast-fed:				
1 month or less wholly	1543	1855	3398	
,, ,, partly	206	225	431	
			3829	87
1 to 2 months wholly	1162	1355	2517	
,, ,, partly	262	372	634	
			3151	72
2 to 3 months wholly	914	1094	2008	
,, ,, partly	295	304	599	
			2607	60
3 to 6 months wholly	751	881	1632	
,, ,, partly	283	336	619	
			2251	51

Children not breast-fed at first visit—549, or 13 per cent. of total.

TABLE 2

BREAST-FEEDING RECORD OF CHILDREN BORN JANUARY TO JUNE, 1937

	City Inner Circle 12 centres		City Outer Circle 21 centres		Total	
	No.	Per cent.	No.	Per cent.	No.	Per cent.
Cause of abandoning breast feeding:						
1. Illness of mother	262	24	264	23	526	23
2. Anxiety of mother (re progress of child)	303	28	430	37	733	33
3. Mother going to work	224	21	113	10	337	15
4. Other causes	299	27	348	30	647	29
Totals	1088		1155		2243	

number of infants are admitted are situated in the districts where the women were accustomed to go out to work before the war; and, incidentally, where the infant mortality rates were high.

Special comments on Table 3

The table set out shows the following points of interest:

(1) THE PERCENTAGE OF INFANTS EITHER NOT BREAST-FED OR BREAST-FED FOR LESS THAN ONE MONTH. The weaning of these cases took place before the health visitor was able to exert any influence on the result, and therefore occurred under the influence of the doctor or midwife. In 1941 the percentage was 7·9 per cent., and in 1942 it was 7·2 per cent. It is believed that a high percentage of these cases are among the unmarried mothers. There is a comparable figure available for Birmingham. We find that in 1938, 13 per cent. of the infants were not breast-fed at the age of one month, so that the present result is actually much better than in the past. The improvement among the married group is greater still, since there has been a definite increase in illegitimate births.

ILLEGITIMATE BIRTHS. FIRST QUARTER. BORN IN BIRMINGHAM

YEAR	No.
1938	180
1939	178
1940	176
1941	169
1942	239

(2) THE TOTAL BABIES FED UP TO, AND OVER, THREE MONTHS.

1941 .. 53 per cent. 1942 .. 52 per cent.

This figure shows no material difference from the results obtained in the 1938 enquiry, when it was found that 51 per cent. of the children were still on the breast from three to six months. The above figures suggest that more breast-feeding is being carried out at the present time than before the war. This may be associated with better economic conditions in many homes. The difference between the first and later infants is not very material.

TABLE 3

BREAST-FEEDING INVESTIGATION, 1942

FIRST QUARTER OF 1941: Total number investigated, 1937.

	Per cent. of 1st or later children	No. of children	Working in pregnancy					Anxious to return	Mother working after confinement				
			Up to (No. of weeks)			Over 32 weeks	Total		1st month	2nd month	3rd month	later	Total
			24	28	32								
Not breast-fed or for less than 1 month:													
First baby	10	100	10	1	—	1	12	8	1	1	3	7	12
2nd or later children ..	5·4	51	5	—	—	—	5	5	—	4	—	2	6
Breast-fed up to 1 month:													
First baby	27	274	28	13	7	5	53	22	6	3	9	23	41
2nd or later children ..	22	207	14	7	2	1	24	19	2	1	3	12	18
Breast-fed up to 2 months:													
First baby	13	129	21	8	4	6	39	13	—	1	6	8	15
2nd or later children ..	15	141	9	9	1	1	20	9	—	5	1	10	16
Breast-fed up to 3 months:													
First baby	11	114	23	9	1	—	33	10	—	—	4	10	14
2nd or later children ..	11·5	108	11	1	1	—	13	5	—	1	—	9	10
Breast-fed over 3 months:													
First baby	38	384	46	23	11	2	82	18	1	4	4	47	56
2nd or later children ..	45	429	18	11	2	4	35	24	—	2	13	45	60
Total investigated:													
Total first babies ..	1001	1937	185	82	29	20	316	133	40	22	43	173	248
,, later babies ..	936												12 per cent.

FIRST QUARTER OF 1942: Total number investigated 2698 cases.

Not breast-fed or for less than 1 month:													
First baby	8	111	9	8	—	—	17	9	1	7	2	3	13
2nd or later children ..	6·4	85	2	2	2	2	8	6	1	7	2	2	12
Breast-fed up to 1 month:													
First baby	29	390	52	24	10	9	95	31	5	16	12	20	53
2nd or later children ..	24·6	324	17	7	12	7	43	17	5	12	3	24	44
Breast-fed up to 2 months:													
First baby	14·2	196	52	23	4	1	80	22	2	5	6	17	30
2nd or later children ..	14·3	189	20	10	3	5	38	18	1	10	3	8	22
Breast-fed up to 3 months:													
First baby	10·3	142	24	13	5	1	43	24	—	2	5	8	15
2nd or later children ..	11·7	155	11	7	2	—	20	12	—	—	2	13	15
Breast-fed over 3 months:													
First baby	39	543	83	35	13	4	135	34	1	2	7	46	56
2nd or later children ..	42·7	563	39	23	8	8	78	39	3	1	10	42	56
Total investigated:													
Total first babies ..	1382	—	—	—	—	—	—	—	—	—	—	—	—
,, later babies ..	1316	2698	309	152	59	37	557	212	19	62	52	183	316
													11 per cent.

Only fifteen cases were definitely quoted in which a mother who had not breast-fed her first infant was successful with a later child. One would, of course, expect some improvement with second and subsequent children.

(3) WORK IN RELATION TO BREAST-FEEDING. It is surprising to find the small percentage (16 per cent.) of women actually working during pregnancy. This percentage is further decreased when the figures for the earlier weeks are omitted. Two per cent. only were working after twenty-eight weeks' pregnancy. The influence of the work during pregnancy can be considered to have little effect on the final totals.

(4) WEANING DUE TO ANXIETY TO RETURN TO WORK. It was repeatedly emphasized that this was mainly among the unmarried mothers.

(5) WEANING THE CHILD IN ORDER TO RETURN TO WORK. Only a comparatively low percentage return to work after confinement until the child is over the age of six months. It is rather that when the child is weaned the mother thinks of working. As long as the child is thriving on the breast, she does not consider the question seriously.

The report of Dr. Pierce is concerned with 164 babies, brought to the casualty and out-patient department of the Children's Hospital. The contrast between these, and the welfare department details, emphasizes the fact that these figures are taken in a specially selected group of the baby population. In view of this, independent comment is made in Dr. Pierce's cases.

The following is an analysis of an enquiry regarding the duration of breast-feeding in babies brought to the out-patient department of the Birmingham Children's Hospital during September, 1942. It is submitted in spite of the small number of cases, since it represents a different cross section of the baby population of the city than the welfare group.

1. Total number of babies, included in the survey—164

1st born	80
2nd born	39
3rd or later sibling	45
Total	164

2. Selection of cases. Consecutive babies under one year of age, seen in the casualty office, and in the surgical clinic, were included in the survey. They fall into two groups, the 'sick' and the 'well' groups, and the findings are analysed with this in mind. The 'sick' babies are those whose presenting complaint was such that it affected the general health and nutrition. The 'well' group are those whose complaint did not affect their general well-being.

(A) DIAGNOSIS:

Sick Babies	No. of cases	Well Babies	No. of cases
Gastro-enteritis	39	Skin. Naevus	49
Upper respiratory infections and otitis	21	Phimosis	26
Skin infections	10	Club foot	9
Whooping cough	2	Hernia	4
Abscesses	2	Total	88
Nephritis	1		
Anaemia	1		
Total	76		

(B) AGE (AT DATE OF INTERVIEW):

(i) Actual average age:

88 Well babies	4 months 27 days
76 Sick babies	4 months 4 days
164 Total group	4 months 15·5 days

(ii) RANGE (IN MONTHS)

Age	Well babies No.	Well babies Percentage	Sick babies No.	Sick babies Percentage
Birth:				
2 weeks	—	—	—	—
2–4 weeks	2	2·27	3	3·94
1–2 months	6	2·81	17	22·36
2–3 months	15	17·04	11	14·47
Total no. and per cent. under 3 months	23	26·12	31	40·77
3–4 months	11	12·50	8	10·50
4–5 months	17	19·31	7	9·21
5–6 months	8	9·09	7	9·21
Total no. and per cent. under 6 months	59	67·02	53	69·71
6–9 months	18	20·45	11	14·47
Total no. and per cent. under 9 months	77	87·47	64	84·18
9–12 months	11	12·50	12	15·79
Total no. and per cent. under 1 year	88	999·7	76	99·97

Among the sick infants, 40 per cent. were under 3 months, 69·71 per cent. under 6 months; the number over 6 months was small, equalling 30 per cent. of the whole. As compared with well babies, the proportion is similar over six months, but under three months there is a marked preponderance of sick babies; this is equalized in a three-to-six-month group.

3. Duration of breast-feeding.

TIME AT WHICH BABY WAS WEANED FROM BREAST

Age when weaned	Well babies No.	Well babies Per cent.	Well babies Per cent.	Sick babies No.	Sick babies Per cent.	Sick babies Per cent.
Never put to breast	11	12·50	—	8	16·52	—
2 weeks or earlier	5	5·69	—	7	9·21	—
	—	—	18·18	—	—	19·73
2 to 4 weeks	—	15·90	—	22	28·94	—
	—	—	34·08	—	—	48·67
1–2 months	17	19·23	—	18	23·68	—
2–3 ,,	11	12·5	—	6	7·89	—
Per cent. weaned by end of 3 months	—	—	65·81	—	—	80·24
3–4 months	10	11·36	—	3	3·94	—
4–5 ,,	8	9·09	—	4	5·26	—
5–6 months	3	3·40	—	4	5·26	—
Per cent. weaned by end of 6 months	—	—	89·86	—	—	94·68
6–9 months	3	3·40	—	2	2·63	—
Per cent. weaned by end of 9 months	—	—	93·26	—	—	97·31
9–12 months	6	6·81	—	2	2·63	—
Per cent. weaned by end of 1 year	88	—	99·86	76	—	99·94

It will be seen that an equal proportion of sick and well babies were weaned in the first fortnight. The next fortnight sees the sick babies leading by 14 per cent. This continues till the end of the third month. The proportion thereafter is equalized.

4. Comparison of duration of breast-feeding in groups of babies with gastro-enteritis and sick babies with other complaints.

(a) Total number of babies with gastro-enteritis 39
,, ,, sick babies with other complaints 37
Total number of sick babies .. 76

Age: Actual average age—gastro-enteritis group, 1 month 3 days.
Actual average age, other sick babies, 1 month 21 days.

Age at date of interview	Gastro-enteritis group			Other sick babies		
	No.	Per cent.	Per cent.	No.	Per cent.	Per cent.
0–2 weeks ..	0	—	—	0	—	—
2–4 ,, ..	0	—	—	3	8·10	—
Per cent. under age one month	—	—	—	—	—	8 10
1–2 months ..	10	25·64	—	7	18·91	—
2–3 ,, ..	10	25·64	—	1	2·70	—
Per cent. under age 3 months	—	—	51·28	—	—	29·71
3–4 months ..	2	5·12	—	6	16·21	—
4–5 ,, ..	4	10·25	—	3	8·10	—
5–6 months ..	3	7·68	—	4	10·81	—
Per cent. under age 6 months	—	—	74·33	—	—	64·83
6–9 months ..	4	10·25	—	7	18·91	—
Per cent. under age 9 months	—	—	84·58	—	—	83·74
9–12 months ..	6	15·39	—	6	16·21	—
	39	—	99·97	37	—	99·95

The higher proportion of young infants suffering from gastro-enteritis, as against other conditions, is evident. This preponderance is lost after 6 months.

(b) Duration of breast-feeding (76 sick children)

TIME AT WHICH BABY WAS WEANED

Age when weaned	Gastro-enteritis group			Other sick baby group		
	No.	Per cent.	Per cent.	No.	Per cent.	Per cent.
2 weeks or less	8	20·51	—	7	18·91	—
2–4 weeks ..	13	33·34	—	9	24·32	—
Per cent. weaned by end of first month ..	—	—	53·85	—	—	43·23
1–2 months ..	10	25·64	—	8	21·62	—
2–3 ,, ..	4	10·25	—	2	5·40	—
Per cent. weaned by end of third month ..	—	—	89·74	—	—	70·25
3–4 months ..	—	—	—	3	8·10	—
4–5 ,, ..	1	2·56	—	3	8·10	—
5–6 ,, ..	1	2·56	—	3	8·10	—
Per cent. weaned by end of six months ..	—	—	94·86	—	—	94·55
6–9 months ..	2	5·12	—	—	—	—
Per cent. weaned by end of ninth month ..	—	—	99·98	—	—	—
9–12 months ..	—	—	—	2	5·40	—
	39	—	99·98	37	—	99·95

This table shows that gastro-enteritis develops in a higher proportion (20 per cent.) of babies weaned early. This preponderance disappears after the sixth month, when the two groups are approximately equal.

5. Employment of mothers

(a) Employment during pregnancy:

Total no. of mothers 164

	No.	Per cent.
No. employed up to 24th week of pregnancy	12	7·4
,, ,, ,, 28th week of pregnancy	6	3·6
,, ,, ,, 32nd week of pregnancy and later ..	11	6·7
Total number employed after six months of pregnancy	29	17·0

(b) Employment of mother during first six months after birth of child.

Mothers returning to work before child was:

2 weeks	1
1 month	0
2 months	1
3 ,, ...	2
4 ,, ...	1
5 ,, ...	3
6 ,, ...	2

Total returning before child was —
six months of age .. .. 10 or 6·07 per cent.

Comments

1. The duration of breast-feeding in this group is considerably shorter than in the welfare group. In this group, 34·19 per cent. of the well babies and 19·76 per cent. of the sick babies were breast-fed after the third month, as compared with 49·5 per cent. in the welfare group.

2. It is significant that babies in the sick group were weaned earlier than in the well group; 48 per cent. were weaned by the end of the first month, and 80 per cent. by the end of the third month, as compared with the well group, in which 34 per cent. were weaned by the end of the first month, and 45 per cent. by the end of the third month. After the sixth month, there is no striking difference in the weaning period of the two groups.

3. The 'sick baby' is younger than the 'well baby' group, but there is no evidence that the onset of symptoms of the current illness played a rôle in the period of weaning.

4. The gastro-enteritis group were weaned somewhat earlier than either the 'well group' or the babies ill with other complaints; 53 per cent. were weaned by the end of the third month, as compared with 43 per cent. of other sick babies weaned at the same period.

5. The percentage of mothers employed during pregnancy was similar to the figure in the welfare group, i.e. 17 per cent. in the former, as compared with 16·3 per cent. in the latter.

6. The number of mothers employed during the early months after the baby's birth was lower (6 per cent. as compared with 12·8 per cent.).

Conclusions

1. Early weaning is related to the occurrence of gastro-enteritis.

2. Early weaning is associated with early infections.

3. There is no suggestion from these figures that weaning is associated with the mother's employment in Birmingham.

4. The usual reason given for weaning is milk failure.

5. The observer gained the impression that the large majority of mothers in both groups were in poor general

health, with pallor, caries, poor skin tone, nervousness, and fatigue. The observer has had a wide experience with a similar group in Chicago, and finds the lower standard of maternal general health is striking, but does not conclude that war causes are the chief factor.

War-time day nurseries

Table shows the observations, made in 178 individual enquiries.

DAY NURSERIES (OCTOBER, 1942)

Babies weaned for the following reasons:

	Total No.
Milk failed—59 per cent.	85
Weaned to allow mother to work—22 per cent.	33
Illness of mother	3
No reason given	7
Doctor's orders	6
Breast abscess	6
Illness of baby	2
Unmarried mothers *	2
Total	144
No report	34
Grand total	178

* Baby not usually in war-time nursery.

It will be noted that a high percentage stated 'milk failed.' This would seem to indicate difficulties in the breast-feeding. Of those weaned to allow the mother to work, the information must be taken in conjunction with the rising number of unmarried mothers. Although only two mothers are noted as unmarried, the fact that a child is illegitimate is not necessarily known at the nursery.

Final conclusions

1. In our opinion, war-time conditions have not materially altered the incidence of breast-feeding.

2. It is evident that very few women are prepared to leave their home to go to work while the baby is under the age of six months.

3. The investigation emphasizes that only 50 per cent. of women are prepared to continue breast-feeding their baby after three months.

SUMMARY OF TABLE 3

Total number of cases investigated:

First quarter of 1941	1937
,, ,, 1942	2698
Grand total investigated	4635
Previous child not breast-fed, but successful with later child	15
1941 Total not breast-fed, or breast-fed for less than 1 month	7·9 per cent.
1942 Total not breast-fed, or breast-fed for less than 1 month	7·2 ,,

Total 1st babies fed up to and over 3 months—

	1941	1942
	498—49·7 per cent.	685—49·5 per cent.
Total 2nd—	537—57·3 per cent.	718—54·5 per cent.
Total	1035—53·4 per cent.	1403—52 per cent.

Working in	1941	1942
Before confinement	16·3 per cent.	20·6 per cent.
After confinement	12·8 ,,	11·7 ,,
Anxious to work	6·8 ,,	7·8 ,,

DAY NURSERIES: Babies under 6 months in attendance at day nurseries in 2nd quarter of 1942:

Total	178
Of these:	
Under 2 months of age	18
Between 2 and 3 months	29
Between 3 and 4 ,,	33
Between 4 and 4 ,,	98
Total	178

Only two mothers out of this number have attended the nursery to breast-feed their babies.